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(54) Title: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

(57) Abstract: The present invention relates to peptides capable of modulating the function (e.g., signaling or adhesive activities) of CD66 (CEACAM) family members and/or their ligands.

PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

Cross-Referenced to Related Applications

This application claims the benefit of United States Provisional Patent Application Serial Numbers 60/150,791 (filed 26 August 1999) and 60/152,501 (filed 2 September 1999), which are incorporated herein by reference.

Background of the Invention

CD66 family members appear to play a role in a wide variety of normal and pathological processes, including: cancer, embryonic development, bacterial infection, viral infection, inflammation, pregnancy, bile transport, and cell adhesion (1-3). CD66 monoclonal antibodies (mAbs) react with members of the carcinoembryonic antigen (CEA) family (4-13). In the CD terminology, mAbs belonging to the CD66 cluster are classified according to their reactivity with each family member, as indicated by a lower case letter after "CD66" as follows: CD66a, CEACAM-1 or biliary glycoprotein (BGP); CD66b, CEACAM-8 or CGM6; CD66c, CEACAM-6 or NCA; CD66d, CEACAM-3 or CGM1; CD66e, CEA; and CD66f, pregnancy specific glycoprotein (PSG) (13, 14). The CD66 (CEA) gene family belongs to the immunoglobulin (Ig) gene superfamily [for review see (1, 2, 15). Structurally, each of the human CD66 family members contains one amino-terminal (N) domain of 108-110 amino acid residues, homologous to Ig variable domains, followed by a differing number (0-6) of Ig C2-type constant-like domains. The structure of the N-domain is therefore predicted to be a stacked pair of beta-sheets with 9 beta-strands (16).

CD66 family members may potentially function as adhesion molecules (12, 17-30). CD66a, CD66c, and CD66e are capable of homotypic and heterotypic adhesion, as shown by use of recombinant CD66a which undergoes homotypic adhesion as well as heterotypic adhesion with CD66c and CD66e (1, 2, 4-12, 17-32). Data also suggest that CD66a plays a signaling role and

regulates the adhesion activity of CD11/CD18 in human neutrophils (8, 11, 25-27, 33, 34). CD66a, CD66b, CD66c, and CD66d, but not CD66e, are expressed in human neutrophils, where they are "activation antigens" in that their surface expression is increased following neutrophil stimulation with various stimuli. CD66a, CD66b, CD66c, and CD66d mAb binding to the neutrophil surface triggers a transient activation signal that regulates the adhesive activity of CD11/CD18, and increases neutrophil adhesion to human umbilical vein endothelial cells (HUVECs) (8, 11, 25-27, 33, 34).

CD66a is frequently down regulated in colon, prostate, breast, and hepatocellular carcinoma, and colorectal adenomas (35-39). Transfection studies have provided evidence that CD66a may act as a tumor suppressor in models of colon cancer (40) prostate cancer (41, 42) breast cancer (43) and bladder cancer (44). CD66a is also important in bacterial infections, since over 95% of pathogenic N. meningitidis and N. gonorrhea interact with CD66a via Opa proteins, and the binding site for these Opa proteins has been localized to the N-domain of CD66a (45-50). An important property of Opa proteins is the stimulation of adhesion and non opsonic phagocytosis of Opa+ bacteria by neutrophils (reviewed in 48). CD66a also appears to function as a receptor for murine hepatitis virus (51-55). Furthermore, CD66a may play a role in angiogenesis since it is selectively expressed on certain endothelial cells (56) and CD66a appears to function as a regulator of bile transport in bile canaliculi (3, 57, 58).

The mechanism(s) by which CD66a transmits signals (e.g. activation in neutrophils, or growth regulating signals in epithelial cells and carcinomas) are unclear. However, CD66a is phosphorylated on its cytoplasmic domain, largely on tyrosine with a lower level of phosphoserine, in neutrophils and colon cancer cells (4, 59-61). While at least eight isoforms of CD66a derived from differential splicing have been described (3, 12, 13, 25), only those isoforms with a long cytoplasmic tail can be phosphorylated on tyrosine. In addition, associated protein tyrosine kinase and phosphatase activities may be involved in CD66a signaling (59, 62, 63).

Summary of the Invention

The present invention relates to peptides capable of modulating the function (e.g., signaling or adhesive activities) of CD66 (CEACAM) family members and/or their ligands. The sequences of these peptides are set forth in Tables I-IX. Active peptides (i.e., those capable of modulating the function of at least one CD66 family member and/or at least one ligand thereof) could be larger or smaller than the ones described here. While the present peptides described are of about 14 amino acids, peptides containing a relatively large number of amino acid residues, e.g., up to about 100 amino acid residues or more, that contain the described peptides, portions thereof, or similar peptides may have biological activity as well. Similarly, peptides smaller than those shown in Tables I-IX may also have similar biological activity. Similarly, peptides with amino acid substitutions or other alterations may block the activities of the described peptides or the parent molecules. Cyclic or otherwise modified forms of the peptides would also be expected to have biological activity.

Thus, the present invention provides isolated peptides that include an amino acid sequence represented by SEQ ID NOs:1-100 or analogs thereof that modulate the function of at least one CD66 protein (i.e., CD66 family member) and/or at least one ligand thereof. These amino acid sequences can form a part of a larger peptide. Additionally, they can be used in various combinations in any one peptide. Preferably, the present invention provides isolated peptides represented by SEQ ID NOs:1, 2, 3, 4, 7-15, 17, 22, 32, 33, 35, 37, 39, 41, 47, 53, or 54. It is believed that SEQ ID Nos:119, 143, 157, 161, 178, and 187 would have activity if they were solubilized or conjugated in a complex.

A preferred group of isolated peptides include those having an amino acid sequence represented by SMPFN (SEQ ID NO:101), PQQLF (SEQ ID NO:102), LPQQL (SEQ ID NO:103), QQLFG (SEQ ID NO:104), NRQIV (SEQ ID NO:105), GNRQI (SEQ ID NO:106), IKSDLVNE (SEQ ID NO:107), AASNPP (SEQ ID NO:108), NTTYLWWVNG (SEQ ID NO:109), YLWWVNG (SEQ ID NO:110), SWLIN (SEQ ID NO:111), SWFIN (SEQ ID NO:112), AQYSWLIN (SEQ ID NO:113), AQYSWFIN (SEQ ID NO:114),

SWFVN (SEQ ID NO:115), AQYSWFVN (SEQ ID NO:116), NRQII (SEQ ID NO:199), GNRQI (SEQ ID NO:200), or analogs thereof. It is believed that these portions of certain of the peptides described herein contribute significantly to the activity of the peptides.

The present peptides are preferably capable of altering signaling mediated in part by CD66 (CEACAM) family members. Preferably, the peptides of the present invention modulate at least one of the following (which are functions of CD66 proteins and/or ligands thereof): activation of neutrophils; activation or inhibition of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells; proliferation and/or differentiation of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells; proliferation and/or differentiation of epithelial cells such as breast or intestinal/colonic epithelium cells or keratinocytes. In addition these peptides are preferably capable of altering homotypic and/or heterotypic adhesion among CD66 proteins (i.e., CD66 family members) or adhesion of CD66 proteins to other CD66 ligands.

The present invention also provides peptide conjugates. The ability of peptides complexed with carrier molecules or structures, such as microbeads, liposomes, biological carrier molecules, synthetic polymers, biomaterials, and cells, thereby forming peptide conjugates is shown to impart the larger structure with the ability to bind to cells expressing the appropriate CD66 family member. Such peptide conjugates bind to cells expressing a CD66 protein or a CD66 ligand.

The peptides or peptide conjugates of the present invention can also include molecules for labeling (i.e., labels such as fluroescence tags, magnetic resonance tags, radioactive tags, enzymatic tags). In this way, these can be used in diagnostic methods to detect specific targets *in vivo* or *in vitro*.

The present invention provides a method of activating a neutrophil that includes contacting the neutrophil with a peptide or peptide conjugate (i.e., at least one peptide or peptide conjugate) that includes an amino acid sequence represented by SEQ ID NOs:1, 2, 3, 4, 17, 41, or analogs thereof. Preferably, the peptide is represented by SEQ ID NOs:1, 2, 3, 4, 17, or 41.

The present invention also provides a method of modulating the homotypic and/or heterotypic adhesion of CD66 family members or adhesion of a CD66 protein to a CD66 ligand. The method includes contacting CD66 family members and/or their ligands with a peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, 54, or analogs thereof. Preferably, the peptide is represented by SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, or 54.

The present invention also provides a method of modulating (e.g., activating or inhibiting) immune cell (e.g., T-cells, B-cells, NK cells, LAK cells, or dendritic cells) activation, proliferation, and/or differentiation that includes contacting an immune cell with a peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:14, 53, or analogs thereof. Preferably, the peptide is represented by SEQ ID NOs:14 or 53.

In addition, some peptides differ from these peptides by one or several amino acids and could compete with these active peptides or the natural CD66 family member or ligand thereof for certain biological activities.

For example, the present invention provides a method of blocking the activation of a neutrophil induced by the method described above. This method includes contacting the neutrophil when in the presence of at least one of the peptides used in the method of activating a neutrophil discussed above with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs: 18-21, 28-31, 39, 40, 55-59, 68-71, 84, or analogs thereof. Preferably, the peptide is represented by SEQ ID NOs:18-21, 28-31, 39, 40, 55-59, 68-71, or 84.

As another example, the present invention provides a method of altering the modulation of the homotypic and/or heterotypic adhesion of CD66 family members or adhesion between a CD66 protein and a CD66 ligand induced by peptides homologous to (e.g., peptides derived from similar regions of similar domains of CD66 family members) those listed above (SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, or 54). The method includes contacting CD66 family members and/or ligands thereof with a peptide comprising an amino acid sequence represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, 72-100, or analogs thereof, when in

the presence of at least one of the peptides listed above (SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, or 54). Preferably, the peptide is represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, or 72-100. This modulating effect can result, for example from direct binding of one of these peptides to one of the CD66 family members and/or ligands thereof, or from altering the effects of other peptides on the adhesion.

Another method of the present invention involves modulating at least one of the following functions of CD66 family members and/or ligands thereof in cells: activation of neutrophils; activation or inhibition of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells; proliferation and/or differentiation of T-cells, B-cells, LAK cells, NK cells, dendritic cells, or other immune system cells; proliferation and/or differentiation of epithelial cells; homotypic and/or heterotypic adhesion among CD66 family members; and adhesion of CD66 family members to other ligands. The method includes contacting cells with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Another method involves delivering a therapeutically active agent to a patient. The method includes administering at least one peptide conjugate comprising a peptide and the therapeutically active agent to a patient wherein the peptide includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof. Preferably, the therapeutically active agent is selected from drugs, DNA sequences, RNA sequences, proteins, lipids, and combinations thereof. More preferably, the therapeutically active agent is an antibacterial agent, antiinflammatory agent, or antineoplastic agent.

There is also provided a method of modifying the metastasis of malignant cells. This method includes contacting the malignant cells or normal host tissue with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

There is also provided a method of altering bacterial or viral binding to cells or a biomaterial. The method includes contacting the cells or biomaterial with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Another method involves altering cell adhesion to a biomaterial. The method includes contacting the biomaterial with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Another method involves detecting tumors. The method includes contacting tumor cells or tumor vasculature with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Still another method involves detecting inflammation. The method includes contacting inflamed vasculature or leukocytes with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Yet another method involves detecting a CD66 protein or a ligand thereof. The method includes contacting tissue containing a CD66 protein or a ligand thereof with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Another method involves altering angiogenesis. The method includes contacting endothelial cells, tumor cells, or immune cells with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Yet another method of the present invention involves altering an immune response. The method includes contacting immune system cells with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Another method of the present invention involves altering keratinocyte proliferation. The method includes contacting keratinocytes with at least one

peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

The methods described herein can be carried out *in vitro* or *in vivo*. The peptides can be used alone or in various combinations as well as in peptide conjugates. They are used in amounts that provide the desired effect. These amounts can be readily determined by one of skill in the art. Preferably, for each of the methods of the present invention, useful peptides are represented by SEQ ID NOs:1, 2, 3, 4, 7-15, 17, 22, 32, 33, 35, 37, 39, 41, 47, 53, or 54.

As used herein, "a" or "an" refers to one or more of the term modified. Thus, the compositions and methods of the present invention include one or more polypeptides. Also, herein when peptide is said to includes an amino acid sequence represented by SEQ ID NOs:1-100 or analogs thereof, the peptide can include one or more of the sequences specified.

"Amino acid" is used herein to refer to a chemical compound with the general formula: NH_2-CRH-COOH, where R, the side chain, is H or an organic group. Where R is an organic group, R can vary and is either polar or nonpolar (i.e., hydrophobic). The amino acids of this invention can be naturally occurring or synthetic (often referred to as nonproteinogenic). As used herein, an organic group is a hydrocarbon group that is classified as an aliphatic group, a cyclic group or combination of aliphatic and cyclic groups. The term "aliphatic group" means a saturated or unsaturated linear or branched hydrocarbon group. This term is used to encompass alkyl, alkenyl, and alkynyl groups, for example. The term "cyclic group" means a closed ring hydrocarbon group that is classified as an alicyclic group, aromatic group, or heterocyclic group. The term "alicyclic group" means a cyclic hydrocarbon group having properties resembling those of aliphatic groups. The term "aromatic group" refers to mono- or polycyclic aromatic hydrocarbon groups. As used herein, an organic group can be substituted or unsubstituted.

The terms "polypeptide" and "peptide" as used herein, are used interchangeably and refer to a polymer of amino acids. These terms do not connote a specific length of a polymer of amino acids. Thus, for example, the terms oligopeptide, protein, and enzyme are included within the definition of polypeptide or peptide, whether produced using recombinant techniques,

chemical or enzymatic synthesis, or naturally occurring. This term also includes polypeptides that have been modified or derivatized, such as by glycosylation, acetylation, phosphorylation, and the like.

Herein, "isolated" as it refers to peptides (i.e., polypeptides) means that the peptides are derived from naturally occurring proteins or other polypeptides and free from other biological material or they are synthesized, either recombinantly or chemically.

The following abbreviations are used throughout the application:

A = Ala = Alanine	T = Thr = Threonine
V = Val = Valine	C = Cys = Cysteine
L = Leu = Leucine	Y = Tyr = Tyrosine
I = Ile = Isoleucine	N = Asn = Asparagine
P = Pro = Proline	Q = Gln = Glutamine
F = Phe = Phenylalanine	D = Asp = Aspartic Acid
W = Trp = Tryptophan	E = Glu = Glutamic Acid
M = Met = Methionine	K = Lys = Lysine
G = Gly = Glycine	R = Arg = Arginine
S = Ser = Serine	H = His = Histidine

Brief Description of the Drawings

Figure 1. Effects of CD66a peptides on neutrophil adhesion to human umbilical vein endothelial cells (HUVECs). HUVECs were grown to confluence in 96 well microtiter plates, and stimulated by adding 50 ng/ml TNF-alpha and 1000 U/ml gamma-interferon (gamma-IFN) and culturing for 48 hours. The wells were then washed and 25 μl of adhesion buffer containing the indicated CD66a peptide at 167 μg/ml (final concentration) was added. One hundred μl of adhesion media containing 10^5 neutrophils labeled with calcein AM was then immediately added, followed by 25 μl of adhesion buffer without (solid bars) or with (hatched bars) 6×10^{-7} M formyl-met-leu-phe (FMLP), and the plates were incubated at 37°C for 30 min in 5% CO_2. The wells were then

washed and the number of adherent neutrophils determined with a fluorescence plate reader. Values are shown as the percent of added neutrophils remaining adherent to the HUVEC monolayers and represent the means +/- SD of 4 separate determinations. The adhesion observed in the presence of the active CD66a peptides CD66a-1, CD66a-2, and CD66a-3 was statistically greater than that observed with 24 other peptides or media alone ($p<0.05$).

Figure 2. Effects of various concentrations of the CD66a peptides CD66a-1, CD66a-2, and CD66a-3 on neutrophil adhesion to HUVECs. HUVECs were grown to confluence in 96 well microtiter plates, and stimulated by incubating in the presence of TNF-alpha at 50 ng/ml final concentration for 4 hr at 37°C, and the adhesion of neutrophils was quantitated in the presence of the indicated final concentration of CD66a peptide CD66a-1 (circles), CD66a-2 (squares), or CD66a-3 (triangles) and 10^{-7} M FMLP as described in Figure 1. Values are shown as the percent of added neutrophils remaining adherent to the HUVEC monolayers and represent the means +/- SD of 4 separate determinations. The adhesion observed in the presence of CD66a peptides CD66a-1, CD66a-2, and CD66a-3 at 50 μg/ml was statistically greater than that observed with lower concentrations of peptides ($p<0.05$).

Figure 3. Effects of scrambled CD66a peptides on neutrophil adhesion to HUVECs. HUVECs were grown to confluence in 96 well microtiter plates, and stimulated by incubating in the presence of TNF-alpha at 50 ng/ml final concentration for 4 hr at 37°C. The wells were then washed and 25 μl of adhesion buffer containing the indicated CD66a peptides (at 167 μg/ml final concentration) was added. One hundred microliters of adhesion media containing 10^5 neutrophils was then added, followed by 25 μl of adhesion buffer without (solid bars) or with (hatched bars) 6 x 10^{-7} M FMLP, and the plates were incubated at 37°C for 30 min in 5% CO_2. The wells were then washed and the number of adherent neutrophils determined with a fluorescence plate reader as in Figure 1. Values are shown as the percent of added neutrophils remaining adherent to the HUVEC monolayers and represent the means +/- SD of 4 separate determinations. The adhesion observed in the presence of the active CD66a peptides CD66a-1, CD66a-2, and CD66a-3, were statistically greater that observed with the 9 scrambled peptides ($p<0.05$).

Figure 4. Representative flow cytometric histogram profiles of the effect of CD66a peptides on human neutrophil surface CD11b and CD62L expression. Left panel: Purified neutrophils were incubated with Hanks' balanced salt solution (HBSS) (mean channel fluorescence (MCF) = 584) (top panel), FMLP (10^{-7} M), (MCF = 709) (second panel), the CD66a peptide CD66a-1 (MCF = 704) (167 μg/ml) (third panel), the CD66a peptide CD66a-2 (MCF = 713) (167 g/ml) (fourth panel), the CD66a peptide CD66a-3 (MCF = 714) (167 μg/ml) (fifth panel), or the scrambled CD66a peptide CD66a-1-S1 (MCF = 581) (167 μg/ml) (bottom panel) for 15 min at 37°C, and the binding of a phycoerythrin-labeled (PE-labeled) CD11b mAb was determined. Vertical axis, relative cell number; horizontal axis, relative fluorescence intensity measured on a log scale. The MCFs represent the means of two determinations that agreed within 10%. Right panel: Purified neutrophils were warmed to 37°C, incubated for 5 min with HBSS (MCF = 548) (top panel), FMLP (10^{-7} M), (MCF = 256) (second panel), the CD66a peptide CD66a-1 (MCF = 230) (167 μg/ml) (third panel), the CD66a peptide CD66a-2 (MCF = 243) (167 μg/ml) (fourth panel), the CD66a peptide CD66a-3 (MCF = 229) (167 μg/ml) (fifth panel), or the scrambled CD66a peptide CD66a-1-S1 (MCF = 546) (167 μg/ml) (bottom panel), and the binding of a PE-labeled CD62L mAb was determined. Vertical axis, relative cell number; horizontal axis, relative fluorescence intensity measured on a log scale. A duplicate experiment gave similar results.

Figure 5. Effects of CD66a-7 peptide on neutrophil adhesion to HUVECs. HUVECs were grown to confluence in 96 well microtiter plates, and stimulated by adding 50 ng/ml TNF-alpha and culturing for 48 hours. The wells were then washed and 25 μl of adhesion buffer with or without the CD66a-7 peptide at 167 μg/ml (final concentration) was added. One hundred microliters (μl) of adhesion media containing 10^5 neutrophils was then immediately added, followed by 25 μl of adhesion buffer with 6×10^{-7} M FMLP, and the plates were incubated at 37°C for 30 min in 5% CO_2. The wells were then washed and the number of adherent neutrophils determined with a fluorescence plate reader. Values are shown as the percent of added neutrophils remaining adherent to the HUVEC monolayers and represent the means +/- SD of 4 separate

determinations. The adhesion observed in the presence of the peptide CD66a-7 was statistically greater than that observed with buffer alone ($p<0.05$).

Figure 6. Effects of CD66a-6L peptide on neutrophil adhesion to HUVECs. Using the method described with respect to Figure 5, the adhesion observed in the presence of the peptide CD66a-6L was statistically greater than that observed with buffer alone ($p<0.05$).

Figure 7. Effects of CD66e-3 peptide on neutrophil adhesion to HUVECs. Using the method described with respect to Figure 5, the adhesion observed in the presence of the peptide CD66e-3 was statistically greater than that observed with buffer alone ($p<0.05$).

Figure 8. Effects of CD66a peptides on binding of CHO transfectants expressing CD66a (CEACAM1-4L) to immobilized recombinant human CEACAM1-Fc using the technique of the Transfectant Binding Assay #1 (Assay #1). Ninety-six well Immulon 3 plates were coated with goat anti-human Fc, washed, and soluble CEACAM1-4-Fc (CD66a-Fc), or the negative control constructs CD31(D1-3)-Fc and CD14-Fc were added and allowed to bind, and the plates were then washed. CHO transfectants were labeled with the fluoresecent tag BCECF-AM and allowed to adhere to these immobilized soluble constructs for 60 min at 37°C. The total fluorescence of each well was then determined using the Cytofluor II fluorescence plate reader. The plates were then washed and the number of cells adhering determined by fluorescence measurements in the Cytofluor II as a percentage of the total cells added per well. The mean +/- SD of 4 determinations are shown. Four of the peptides, CD66-17, CD66-18, CD66-19, and CD66-24, significantly inhibited homotypic CD66a binding in this assay.

Figure 9A and 9B. Effects of peptides on homotypic adhesion of CD66a-CD66a using Transfectant Binding Assay #2 (Assay #2). Several peptides blocked binding of CD66a expressing CHO transfectants to immobilized CD66a using the technique of Assay #2. The data is shown as percent of added cells that remained adherent. Control values "0" and "1000" represent the adhesion observed when no CD66a protein is adherent to the well, or when buffer is added without peptide, respectively. Control values "0.01" represent the adhesion observed in the presence of a blocking CD66 antibody.

Figure 10A and 10B. Effects of peptides on homotypic adhesion of CD66c-CD66c using Assay #2. Several peptides blocked binding of CD66c expressing CHO transfectants to immobilized CD66c using the technique of Assay #2. The data is shown as percent of added cells that remained adherent. Control values "0" and "1000" represent the adhesion observed when no CD66c protein is adherent to the well, or when buffer is added without peptide, respectively. Control values "0.01" represent the adhesion observed in the presence of a blocking CD66 antibody.

Figure 11A and 11B. Effects of peptides on homotypic adhesion of CD66e-CD66e using Transfectant Binding Assay #2 (Assay #2). Several peptides blocked binding of CD66e expressing CHO transfectants to immobilized CD66e using the technique of Assay #2. The data is shown as percent of added cells that remained adherent. Control values "0" and "1000" represent the adhesion observed when no CD66e protein is adherent to the well, or when buffer is added without peptide, respectively. Control values "0.01" represent the adhesion observed in the presence of a blocking CD66 antibody.

Figure 12A and 12B. Effects of peptides on heterotypic adhesion of CD66b-CD66c using Assay #2. Several peptides blocked binding of CD66b expressing CHO transfectants to immobilized CD66c using the technique of Assay #2. The data is shown as percent of added cells that remained adherent. Control values "0" and "1000" represent the adhesion observed when no CD66c protein is adherent to the well, or when buffer is added without peptide, respectively. Control values "0.01" represent the adhesion observed in the presence of a blocking CD66 antibody.

Figure 13A and 13B. Binding of microbeads coupled to CD66a-24 to CHO cells expressing CD66a. CD66a-24 and CD66a-1 peptides were coupled to microbeads and the microbeads were incubated with a suspension of CHO cells expressing CD66a at room temperature for 30 min. The binding of the microbeads to the CHO cells was quantified by counting the number of beads associated with single cells or groups of cells in three cell-group size classes and are reported as the number of microbeads bound to each size group of cells. Figure 13B shows the number of beads associated with single cells, which are reported as the average number of microbeads bound to each single cell.

Figure 14. Effect of CD66 peptides on the activation of T-cells. T-cells were stimulated with anti-CD3 in the presence of various CD66 peptides as indicated and proliferation quantitated using radionucleide uptake expressed as cpm associated with the cells. Peptide CD66a-24, and to a lesser extent CD66e-31, inhibited T-cell activation.

Detailed Description of Preferred Embodiments of the Invention

Because of the adhesive and signaling properties of CD66a described above, we sought to identify functionally active domains of CD66a by use of synthetic peptides. Peptides of 14 amino acids in length were synthesized. The sequences are set forth in Tables I-IX. These were investigated for the ability to modulate the function of CD66 (CEACAM) family members. Thus, the present invention provides isolated peptides that include an amino acid sequence represented by (at least one of) SEQ ID NOs:1-100 or analogs thereof that modulate the function of at least one CD66 protein (i.e., CD66 family member) and/or at least one ligand thereof.

Peptides were tested for their ability to alter neutrophil adhesion to human umbilical vein endothelial cells (HUVECs). Five peptides activated neutrophils for adhesion to endothelial cells, as determined by increasing neutrophil adhesion to HUVEC monolayers and altering surface expression of CD11/CD18 and CD62L. The data suggest that at least 5 peptide motifs from the N-terminal domain of CD66a are involved in the interaction of CD66a with other ligands, and can initiate signal transduction in neutrophils. These 5 motifs have the amino acid sequences represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:17. Activating or inhibiting neutrophil activation may be useful in treating certain infectious diseases or in cases where the activation of neutrophils results in unwanted effects as in adult respiratory distress syndrome.

Similar modeling was done with CD66b, CD66c, CD66d, CD66e, and CD66f and peptides shown in Tables III-VIII were synthesized. One of these peptides was also found to activate neutrophils. This peptide has the sequence represented by SEQ ID NO:41. In addition, it was found that peptides from homologous regions of other CD66 members that contained minor amino acid

differences from the active peptides from CD66a did not activate neutrophils, thus providing further information on the structure needed for activity. These include peptides having the amino acid sequences represented by SEQ ID NOs:18-21, 28-31, 39, 40, 55-59, 68-71, and 84. These peptides could compete with the active peptides (SEQ ID NOs:1-4, 17, and 41) or could mediate direct binding of natural CD66 family members.

These peptides were also tested for their ability to alter the homotypic adhesion of CD66a to CD66a, CD66c to CD66c, and CD66e to CD66e, as well as the heterotypic adhesion of CD66b to CD66c. A number of the peptides were found to modulate homotypic and/or heterotypic adhesion of CD66 family members. These include peptides having the amino acid sequences represented by SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, and 54. It is believed that these may also modulate adhesion between a CD66 protein and other CD66 ligands. In addition, some peptides that differ from these active peptides by one or several amino acids could compete (i.e., alter their modulation effects) with these active peptides for functional effects or mediate direct binding of the natural CD66 family members. These include peptides having the amino acid sequences represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, and 72-84, as well as other homologous peptides (based on domain structure) including SEQ ID NOs:85-100.

Peptides were also tested for their ability to inhibit the activation of T-cells toward proliferation and/or differentiation. One peptide (SEQ ID NO:14) was found to be a potent inhibitor of T-cell activation while another (SEQ ID NO:53) had weaker activity. Modulating the immune response, as for example by activating or inhibiting the proliferation and/or differentiation of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells, may be useful in treating autoimmune diseases, and in transplantation therapies where graft vs. host or host vs. graft effects may be undesirable. The peptides could also be immune stimulants in settings such as cancer, infectious disease, or immunization. Alternatively, they could be immune suppressants. They could also be used to detect inflammation, and preferably modulate inflammation by activating or inhibiting activation of immune or inflammatory

cells. A preferred method involves detecting (and preferably modulating) inflammation in tissues such as inflamed vasculature or leukocytes.

Thus, preferably, the present invention provides isolated peptides represented by SEQ ID NOs:1, 2, 3, 4, 7-15, 17, 22, 32, 33, 35, 37, 39, 41, 47, 53, or 54. It is also believed that peptides represented by SEQ ID Nos:119, 143, 157, 161, 178, and 187 would have activity if they were solubilized or conjugated in a complex.

Thus, the present invention provides peptides derived from CD66 (CEACAM) family members that are capable of modulating (i.e., altering by increasing, decreasing, etc.), for example, cell activation, cell adhesion, cell proliferation, cell differentiation, or homotypic and/or heterotypic adhesion among CD66 family members or binding of CD66 family members to their ligands.

In addition to the peptides discussed above that are specifically shown to have such activity, others are believed to possess a least one activity as described herein. These peptides are shown in Tables I-IX.

Compositions comprising the polypeptides of this invention can be added to cells in culture (*in vitro*) or used to treat patients, such as mammals (*in vivo*). Where the polypeptides are used to treat a patient, the polypeptide is preferably combined in a pharmaceutical composition with a pharmaceutically acceptible carrier such as a larger molecule to promote polypeptide stability or a pharmaceutically acceptable buffer that serves as a carrier for the polypeptide or incorporated in a peptide conjugate that has more than one peptide coupled to a single entity.

Given the known bacterial and viral binding properties of CD66 family members, the peptides described herein could be useful for altering the binding of viruses, bacteria, or other pathological etiologic agents to the cells of host tissues, transplanted tissues, or to biomaterials (increase or inhibit binding). They could also be useful for detecting a CD66 protein or a ligand thereof in tissue, whether it be *in vitro* or *in vivo*.

Studies were also performed to demonstrate that these peptides could be used to target the binding of larger structures to cells expressing the appropriate CD66 family member. The coupling of multiple copies of peptides to larger

structures (thereby forming peptide conjugates) allows cooperativity of binding due to the presence of multiple binding sites. This markedly increases the affinity of binding of the complex compared with that of a single free peptide. In addition, it should therefore be possible to complex various combinations and densities of different peptides described herein to create a structure that preferentially binds cells expressing a specific pattern of CD66 family members.

The biological activity of the peptides identified here suggests that they have sufficient affinity to make them potential candidates for drug localization to cells expressing the appropriate surface structures. This targeting and binding to cells could be useful for the delivery of therapeutically active agents (including targeting drugs, DNA sequences, RNA sequences, lipids, proteins (e.g., human growth factors)) and gene therapy/gene delivery. More preferably, the therapeutically active agent is an antibacterial agent, antiinflammatory agent, or antineoplastic agent.

Since different cells, including specifically many malignant cells, cells of different tissues, growing endothelial cells, including endothelial cells in new vessels in tumors and in diabetic proliferative microvasculature, express different combinations of CD66 family members, it should be possible to generate compounds bearing different combinations of densities of CD66 peptides that would target (bind preferentially) to different desired tissues or cells.

As proof of principle, the peptide CD66-24 when coupled to microbeads directs the binding of the complexed microbeads to CHO cells expressing CD66a.

Also, CD66 family members have been shown to alter metastases of malignant cells and can alter cell differentiation. Thus, the peptides described herein could modify the process of metastasis of malignant cells either by altering the behavior of the malignant cells directly, or by altering the physiology of a target tissue (as for example, the liver where CD66e has been shown to alter cytokine production by cells in the liver and also alter the ability of colon cancer cells to metastasize to the liver). The peptides described herein can also be used in detecting tumors.

Thus, the peptides described herein are believed to be useful for altering angiogenesis. In such a method, endothelial cells, tumor cells, or immune cells are contacted with at least one peptide described herein.

Some CD66 members are expressed in growing keratinocytes at the edge of healing wounds.. These peptides may be useful to alter keratinocyte growth or behavior or the behavior of other cell involved in wound healing.

These peptides may be useful in altering the growth or physiology of cells, which are in various disease states, that can express CD66 members, including gut (as for example in inflammatory bowel disease, atrophic states, or cancer), breast, stomach, small bowel, colon, pancreas, thyroid, prostate, lung, kidney, placenta, sebaceous glands, and uterus.

Treatment for these various conditions can be prophylactic or therapeutic. Thus, treatment can be initiated before, during, or after the development of the condition. As such, the phrases "inhibition of" or "effective to inhibit" a condition includes both prophylactic and therapeutic treatment (i.e., prevention and/or reversal of the condition).

Additionally, molecules/particles with a specific number of specific CD66 peptides would bind specifically to cells/tissues expressing specific ligand combinations, and therefore could have diagnostic and therapeutic use. Thus, the peptides of the present invention can be labeled (e.g., fluorescent, radioactive, enzyme, nuclear magnetic) and used to detect specific targets *in vivo* or *in vitro* including "immunochemistry" like assays *in vitro*. *In vivo* they could be used in a manner similar to nuclear medicine imaging techniques to detect tissues, cells, or other material expressing specific CD66 ligands.

The polypeptides of SEQ ID NOs:1-100 can be in their free acid form or they can be amidated at the C-terminal carboxylate group. The present invention also includes analogs of the polypeptide of SEQ ID NOs:1-100, which typically have structural similarity with SEQ ID NOs:1-100. An "analog" of a polypeptide includes at least a portion of the polypeptide, wherein the portion contains deletions or additions of one or more contiguous or noncontiguous amino acids, or containing one or more amino acid substitutions. Substitutes for an amino acid in the polypeptides of the invention are preferably conservative substitutions, which are selected from other members of the class to which the

amino acid belongs. An analog can also be a larger peptide that incorporates the peptides described herein. For example, it is well-known in the art of protein biochemistry that an amino acid belonging to a grouping of amino acids having a particular size or characteristic (such as charge, hydrophobicity and hydrophilicity) can generally be substituted for another amino acid without substantially altering the structure of a polypeptide.

For the purposes of this invention, conservative amino acid substitutions are defined to result from exchange of amino acids residues from within one of the following classes of residues: Class I: Ala, Gly, Ser, Thr, and Pro; Class II: Cys, Ser, Thr, and Tyr; Class III: Glu, Asp, Asn, and Gln (carboxyl group containing side chains): Class IV: His, Arg, and Lys (representing basic side chains); Class V: Ile, Val, Leu, Phe, and Met (representing hydrophobic side chains); and Class VI: Phe, Trp, Tyr, and His (representing aromatic side chains). The classes also include other related amino acids such as halogenated tyrosines in Class VI.

Polypeptide analogs, as that term is used herein, also include modified polypeptides. Modifications of polypeptides of the invention include chemical and/or enzymatic derivatizations at one or more constituent amino acid, including side chain modifications, backbone modifications, and N- and C-terminal modifications including acetylation, hydroxylation, methylation, amidation, and the attachment of carbohydrate or lipid moieties, cofactors, and the like.

A preferred polypeptide analog is characterized by having at least one of the biological activities described herein. Such an analog is referred to herein as a "biologically active analog" or simply "active analog." The biological activity of a polypeptide can be determined, for example, as described in the Examples Section.

For example, active analogs of SEQ ID NO:1 include peptides having an "M" or similar amino acid in the "SMPFN" sequence (SEQ ID NO:101). Active analogs of SEQ ID NO:2 include peptides having a "Q" or similar amino acid in the "PQQLF" sequence (SEQ ID NO:102), the "LPQQL" sequence (SEQ ID NO:103), or the "QQLFG" sequence (SEQ ID NO:104). Active analogs of SEQ ID NO:3 include peptides having an "RQ" sequence or similar

amino acid sequence in the "NRQIV" sequence (SEQ ID NO:105) or the "GNRQI" sequence (SEQ ID NO:106). Active analogs of SEQ ID NO:4 include peptides having an "IKSDLVNE" portion (SEQ ID NO:107) of the sequence. Active analogs of SEQ ID NO:9 include peptides having an "AASNPP" portion (SEQ ID NO:108) of the sequence. Active analogs of SEQ ID NO:22 include peptides having a "NTTYLWWVNG" portion (SEQ ID NO:109) or "YLWWVNG" portion (SEQ ID NO:110) of the sequence. Active analogs of SEQ ID NO:35 include peptides having an "SWLIN" portion (SEQ ID NO:111), "SWFIN" portion (SEQ ID NO:112), "AQYSWLIN" portion (SEQ ID NO:113), or "AQYSWFIN" portion (SEQ ID NO:114) of the sequence. Active analogs of SEQ ID NO:47 include peptides having an "SWFVN" portion (SEQ ID NO:115) or "AQYSWFVN" portion (SEQ ID NO:116) of the sequence. Active analogs of SEQ ID NO:41 include peptides having an "NRQII" portion (SEQ ID NO:199) or "GNRQI" portion (SEQ ID NO:200).

The polypeptides of the invention may be synthesized by the solid phase method using standard methods based on either t-butyloxycarbonyl (BOC) or 9-fluorenylmethoxy-carbonyl (FMOC) protecting groups. This methodology is described by G.B. Fields et al. in Synthetic Peptides: A User's Guide, W.M. Freeman & Company, New York, NY, pp. 77-183 (1992). The present peptides may also be synthesized via recombinant techniques well known to those skilled in the art. For example, U.S. Patent No. 5,595,887 describes methods of forming a variety of relatively small peptides through expression of a recombinant gene construct coding for a fusion protein which includes a binding protein and one or more copies of the desired target peptide. After expression, the fusion protein is isolated and cleaved using chemical and/or enzymatic methods to produce the desired target peptide.

The peptides of the present invention may be employed in a monovalent state (e.g., free peptide or peptide coupled to a carrier molecule or structure). The peptides may also be employed as conjugates having more than one (same or different) peptide bound to a single carrier molecule. The carrier molecule or structure may be microbeads, liposomes, biological carrier molecule (e.g., a glycosaminoglycan, a proteoglycan, albumin, or the like), a synthetic polymer

(e.g., a polyalkyleneglycol or a synthetic chromatography support), biomaterial (e.g., a material suitable for implantation into a mammal or for contact with biological fluids as in an extrcorporeal device), or other cell. Typically, ovalbumin, human serum albumin, other proteins, polyethylene glycol, or the like are employed as the carrier. Such modifications may increase the apparent affinity and/or change the stability of a peptide. The number of peptide fragments associated with or bound to each carrier can vary. In addition, as mentioned above, the use of various mixtures and densities of the peptides described herein may allow the production of complexes that have specific binding patterns in terms of preferred ligands.

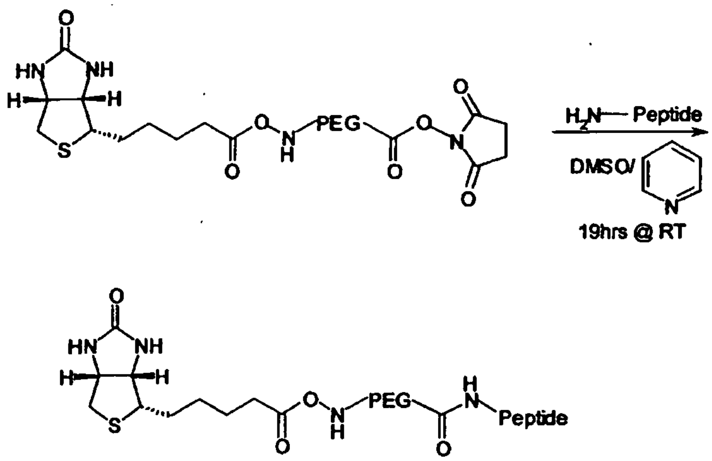
The polypeptides can be conjugated to other polypeptides using standard methods known to one of skill in the art. Conjugates can be separated from free peptide through the use of gel filtration column chromatography or other methods known in the art.

For instance, peptide conjugates may be prepared by treating a mixture of peptides and carrier molecules (or structures) with a coupling agent, such as a carbodiimide. The coupling agent may activate a carboxyl group on either the peptide or the carrier molecule (or structure) so that the carboxyl group can react with a nucleophile (e.g. an amino or hydroxyl group) on the other member of the peptide conjugate, resulting in the covalent linkage of the peptide and the carrier molecule (or structure).

As another example, peptides may be coupled to biotin-labeled polyethylene glycol and then coupled to avidin containing compounds, for instance, as shown in Fig. 13. Peptides are weighed out in aliquots of 0.5 mg and dissolved in a total volume of 500 µl dimethyl sulfoxide (DMSO, FisherChemical, Fair Lawn, NJ) in a 1 mL ReactiVial containing a flea bar. To each ReactiVial, 1.0 mg Biotin-PEG-NHS, average MW 3400, (Shearwater Polymers, Huntsville, AL) is added directly and the vial is moved to a stir plate to provide gentle mixing. Pyridine (Sigma Chemical, St. Louis, MO) is added as a basic catalyst at a 5% molar excess to the peptide. The reaction is allowed to proceed for 19 hours at room temperature with medium stirring.

After completion of the reaction, the contents of each ReactiVial are individually transferred to a 1.5 mL plastic microfuge tube. Each vial is washed

once with 25 µl DMSO which is also added to the microfuge tube. The volume of DMSO is dried down at room temperature to approximately 20 µl of remaining solvent in a Savant Speed Vac Plus. To each tube individually, 980 µl of Hanks balanced salt solution (HBSS) + 0.1% sodium azide is added. Samples are stored at –20°C until coupling to streptavidin-coated beads.



Reaction scheme for boitinylation of peptides.

Streptavidin-coated 6 µm diameter polystyrene beads are obtained from Polysciences (Warrington, PA). For each peptide, 100 µl of suspended beads are aliquoted to a 1.5 ml plastic microfuge tube. As per the manufacturer's directions, the beads are washed three times by sequentially pelleting the beads in a microcentrifuge, decanting the supernatant and redispersing them in 1 ml of fresh phosphate buffered saline (PBS). One third (333 µl) of the biotinylated peptide from the above preparation is added to the beads in a total volume of 1 ml. From the reported binding capacity of the streptavidin-coated beads, this amount of pegylated peptide respresents more than a two-fold molar excess, thus the biotin binding sites are believed to be saturated. The tubes are mixed end-to-end on a rocker plate at 100 revolutions per minute (RPM) for 1 hour. The beads are then washed once as before and resuspended in 1 ml of a 0.1 M ethanolamine solution and mixed on the rocker plate as before for 30 minutes. This step serves to block any potentially unreacted NHS moieties. The beads

are again washed once as before and resuspended in HBSS + 0.1% sodium azide. In the case of peptides coupled to other entities, it should be understood that the designed activity may depend on which end of the peptide is coupled to the entity.

The present invention also provides a composition that includes one or more active agents (i.e., polypeptides) of the invention and one or more pharmaceutically acceptable carriers. One or more polypeptides with demonstrated biological activity can be administered to a patient in an amount alone or together with other active agents and with a pharmaceutically acceptable buffer. The polypeptides can be combined with a variety of physiological acceptable carriers for delivery to a patient including a variety of diluents or excipients known to those of ordinary skill in the art. For example, for parenteral administration, isotonic saline is preferred. For topical administration, a cream, including a carrier such as dimethylsulfoxide (DMSO), or other agents typically found in topical creams that do not block or inhibit activity of the peptide, can be used. Other suitable carriers include, but are not limited to alcohol, phosphate buffered saline, and other balanced salt solutions.

The formulations may be conveniently presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Preferably, such methods include the step of bringing the active agent into association with a carrier that constitutes one or more accessory ingredients.

The methods of the invention include administering to a patient, preferably a mammal, and more preferably a human, the composition of the invention in an amount effective to produce the desired effect.

The peptides can be administered as a single dose or in multiple doses. Useful dosages of the active agents can be determined by comparing their *in vitro* activity and the *in vivo* activity in animal models. Methods for extrapolation of effective dosages in mice, and other animals, to humans are known in the art.

The agents of the present invention are preferably formulated in pharmaceutical compositions and then, in accordance with the methods of the invention, administered to a patient, such as a human patient, in a variety of forms adapted to the chosen route of administration. The formulations include,

but are not limited to, those suitable for oral, rectal, vaginal, topical, nasal, ophthalmic, or parental (including subcutaneous, intramuscular, intraperitoneal, intratumoral, intraorgan, intraarterial and intravenous) administration.

Formulations suitable for parenteral administration conveniently include a sterile aqueous preparation of the active agent, or dispersions of sterile powders of the active agent, which are preferably isotonic with the blood of the recipient. Absorption of the active agents over a prolonged period can be achieved by including agents for delaying, for example, aluminum monostearate and gelatin.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as tablets, troches, capsules, lozenges, wafers, or cachets, each containing a predetermined amount of the active agent as a powder or granules, as liposomes containing the active agent, or as a solution or suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion, or a draught. Such compositions and preparations typically contain at least about 0.1 wt-% of the active agent. The amount of polypeptide (i.e., active agent) is such that the dosage level will be effective to produce the desired result in the patient.

Nasal spray formulations include purified aqueous or other solutions of the active agent with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Formulations for rectal or vaginal administration may be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids. Ophthalmic formulations are prepared by a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye. Topical formulations include the active agent dissolved or suspended in one or more media such as mineral oil, petroleum, polyhydroxy alcohols, or other bases used for topical pharmaceutical formulations.

Examples

Materials and Methods

Cell Preparation. Normal peripheral blood neutrophils were prepared by a modification of the method of Boyum as previously described (64) and were suspended at the indicated concentrations in Hanks' balanced salt solution (HBSS) (Gibco, Grand Island, NY). Differential cell counts on Wright-stained cells routinely revealed greater than 95% neutrophils. Viability as assessed by trypan blue dye exclusion was greater than 98%.

Antibodies and Reagents. The PE-labeled CD11b mAb (Leu 15) and the CD62L mAb (Leu 8) were obtained from Becton Dickenson, Mountain View, CA. Monoclonal antibodies were diluted in PBS containing 1 mg/ml BSA as indicated. N-formyl-met-leu-phe (FMLP) and normal mouse serum (NMS) were purchased from Sigma Chemical Co. (St. Louis, MO). Peptides were diluted in PBS containing 1 mg/ml BSA as indicated.

Fluorescence labeling of cells. Neutrophils were labeled with calcein AM (Molecular Probes, Eugene, OR) (65, 66) by incubating 5 x 10^6/ml cells with 50 μg of calcein AM for 30 min at 37°C in 18 ml of calcein labeling buffer (HBSS without Ca^{2+} or Mg^{2+} containing 0.02% BSA). Cells were then washed twice with calcein labeling buffer at 23°C and resuspended in the desired media.

Endothelial cell adhesion assay. Neutrophil adhesion to human umbilical vein endothelial cells (HUVECs) was determined as previously described (65-68). Briefly, HUVECs (Clonetics Corp., San Diego, CA) were passaged 1:5 in T-25 flasks (Costar) no more than three times before plating in 96 well microtiter plates at 3000 cells/well. HUVECs were grown to confluence in 96 well microtiter plates in EGM media (Clonetics) and fed every 24 hours. Using the adhesion assay described below, no difference in resting and stimulated neutrophil adhesion was observed, and, as expected (69), no difference in surface expression of CD54 (ICAM-1) or CD62E (E selectin, ELAM-1) in resting or TNF stimulated cells was noted using HUVECs passaged once compared with those passaged five times. In some experiments, the HUVECs were stimulated by culture for the indicated time with the desired cytokines (TNF-alpha (Cetus, Emeryville, CA) or gamma-IFN (gift from Dr. S. Palm, University of Minnesota Medical School)). The wells were then washed

four times with adhesion buffer (DMEM + 5% heat inactivated fetal bovine serum (HIFBS)) and 25 µl of adhesion buffer containing the indicated peptide was added to each well, followed immediately by 100 µl of adhesion buffer containing 10^5 calcein labeled cells. Twenty-five microliters of adhesion buffer containing the indicated concentration of FMLP was then added, and the plates were incubated at 37°C in 5% CO_2 for 30 min. The wells were then aspirated and washed four times with endo wash buffer (HBSS + 4% HIFBS), and the fluorescence was quantitated with a Millipore fluorescence plate reader using an excitation wavelength of 485 nm and an emission wavelength of 530 nm. For each condition, quadruplicate wells were tested and values are reported as the mean +/- SD. Each experiment was performed at least four times using different HUVEC subcultures.

Statistical analyses. Effects of peptides on neutrophil adhesion to HUVECs was analyzed by the Mann Whitney U test when appropriate.

Analysis of CD11b and CD62L expression. For analysis of CD11b upregulation, purified neutrophils (10^5 in 100 µl HBSS + 0.02% BSA) were incubated with media containing the indicated peptide (167 µg/ml) or FMLP (10^{-7} M) for 15 min at 37°C. The cells were then cooled to 0°C for 10 min and 2 µg of the PE-labeled CD11b mAb was added. The mixture was incubated at 0°C for 25 min, and 4 ml of buffer B (PBS, pH 7.4, 0.2% BSA, 0.05% NaN3) (0°C) was then added and the mixture was centrifuged at 400 x g for 5 min at 4°C. The supernatant was removed and the cells were vortexed, and suspended in 1 ml of buffer B (0°C), and 250 ml of fixative (Coulter) (23°C) was then added. Three ml of buffer B (0°C) was then added, and the mixture centrifuged at 400 x g at 4°C for 5 min. The cells were washed with 3 ml of buffer B as above, and resuspended in 200 ml of PBS containing 0.1% NaN_3 (0°C) and stored at 4°C until analysis. Quantitative flow cytometric analysis of surface antigen expression was performed using a FACSTAR Plus (Beckton Dickinson, Mountain View, CA). Forward and right angle light scatter, as well as the peak fluorescence channel, were optimized with fluorescent beads. The cell population studied was determined by forward and right angle light scatter.

For analysis of CD62L down regulation, purified neutrophils (10^5 in 100 µl HBSS + 0.02% BSA) were warmed to 37°C for 5 min and then incubated

with media containing the indicated peptide (167 μg/ml) or FMLP (10^{-7} M) for 5 min at 37°C. The cells were then cooled to 0°C for 10 min and 5 μg of the PE-labeled CD62L mAb was added. The cells were then incubated, washed, and analyzed by flow cytometry as above.

Peptide selection, synthesis, and purification. CD66a was modeled to conform to the IgV and Ig C2 domains of the heavy and light chains of Fab fragments of immunoglobulin and CD4.

Peptides were synthesized as amides by Fmoc solid-phase methodology on a Gilson Automated Multiple Peptide Synthesizer AMS 422. Peptides were purified by preparative reverse phase-HPLC on a Beckman System Gold equipped with a Regis Chemical ODS C18 column (10 μm particle size, 60 Angstrom pore size, 250 x 21.1 mm). The elution gradient was 12-50% B over 35 min at a flow rate of 5.0 ml/min, where A is water containing 0.1% trifluoroacetic acid, and B is acetonitrile containing 0.1% trifluoroacetic acid. Detection was at 235 nm. Peptide purity and fidelity can be analyzed by amino acid analysis and sequencing or by mass spectrometry.

Once the first peptides were screened in our adhesion assay (Fig. 1), the amino acids in the positive peptides, CD66a-1, CD66a-2, and CD66a-3, were randomly scrambled and the control peptides were synthesized (Table II). The scrambled amino acid residue peptides were then tested in the same assays in order to ensure that the primary amino acid sequences were essential for the functional activity of these peptides, and that the biological activity was not merely due to the peptides' net charge or amino acid composition (Fig. 3).

Flow cytometry demonstrated that CD66a-1, CD66a-2, and CD66a-3 upregulated CD11b and down regulated CD62L neutrophils (Fig. 4).

We next completed the synthesis of peptide CD66a-7 and found that it has similar activity as CD66a-1 (Fig. 5).

Since peptide CD66a-6 was not soluble we synthesized peptides from the same region but shifted the center of the peptide in an attempt to generate a soluble peptide. One peptide, CD66a-6L, (Table I) was successfully synthesized, tested, and found to stimulate neutrophil adhesion to HUVECs (Fig. 6).

Since only the N-domain peptide of CD66a had activity in the neutrophil activation assay we modeled CD66b, CD66c, CD66d, CD66e, and CD66f N-domains and synthesized appropriate peptides as shown in Tables III-VIII. Of these peptides, only peptide CD66e-3 activated neutrophils (Fig. 7). These results are noteworthy in that many peptides that have only minor differences from active peptides had no biological activity.

<u>Method #1 for adhesion assay of CHO transfectant binding to immobilized recombinant CD66a (Assay #1)</u>. Stable CHO cell transfectants expressing CD66a (CEACAM1-4L) CEACAM1-4S, CEACAM1-1S or the neomycin resistance gene (CHO-Neo) (provided by Dr. S. Watt, MRC, Oxford, UK) were grown to 50-70% confluence in Hams-F10 medium containing 10% (v/v) FBS. Adherent cells were detached with PBS containing 1 mM EDTA pH 7.4, washed three times with Hams-F10 medium, and resuspended in Hams-F10 medium at 2×10^6 cells/ml. The fluorescent dye 2',7'-bis-(2-carboxyethyl)-5-(and –6)-carboxfluoresceinacetoxymethylester (BCECF-AM); Molecular Probes, Eugene, OR) was dissolved in DMSO at 500 μg/ml and 20 μl added per 2×10^7 cells for 20-30 min at 37°C. Cells were washed twice with RPMI-1640 medium and twice with PBS containing 0.2% BSA (PBS-0.2% BSA). Cells (5-10×10^4) in PBS-0.2% BSA were added to 96 well Immulon 3 flat bottomed microtiter plates (Dynatech) that had been pre-coated as follows. Purified goat anti-human Fc antibody (Sigma Chemical Co.) was added to 96 well flat bottomed Immulon 3 plates at 1 μg/100 μl/well at 4°C overnight. The plates were washed 4 times with PBS containing 0.5% BSA (PBS-0.5% BSA) and blocked with PBS-0.5% BSA for at least two hours at room temperature. After washing the plates 4 times with PBS, 50 μl of soluble recombinant protein containing the Fc fragment of human IgG1 attached to CEACAM1 (CEACAM1-Fc) or other indicated protein (10 μg/ml) in PBS were added for at least 2 hours at room temperature or overnight at 4°C. The plates were washed 4 times with PBS before the addition of 100 μl of cells. CHO cell transfectants labeled with BCECF-AM were allowed to adhere for 60 min at 37°C before reading the total BCECF-AM fluorescence in each well on a Cytofluor II plate reader (PerSeptive Biosystems, Hertford, UK) at an excitation wavelength of

485/20 nm, a gain of 70 and an emission wavelength of 530/30 nm. The plates were washed one to three times with PBS-0.2% BSA and the percentage of cells adhering to the constructs estimated from the subsequent fluorescence determinations on the Cytofluor II. Adhesion assays were performed with 4 to 6 replicates in at least two independent experiments.

Method #2 for adhesion assay of CHO transfectant binding to immobilized recombinant CD66a (Assay #2). CHO cells transfected with BGPa cDNA (courtesy Dr. M. Kuroki) were grown in α-MEM (Gibco Inc., Grand Island, NY) lacking nucleosides with 10% FBS (Bio-Whittaker, Walkersville, MD) and antibiotics. Cell cultures were maintained by passing 1:10 in T-25 flasks approximately every 3 days. For the assay, one T-25 of cells near confluence was trypsinized and the collected population was washed once with growth media and resuspended 0.5 mL growth media. To obtain a single-cell suspension, cells were passed sequentially through an 18-gauge, 22-gauge and 25-gauge needle.

One μg of protein in 50 μl of PBS was dried down in a well of a 96-well plate. Wells were incubated with 0.5% BSA in PBS (200 μl/well) for blocking for 4 hours at room temperature. CHO transfectants expressing the appropriate CD66 family member were incubated in serum-free MEM containing 25 μg/ml H33342 dye at 1 x 10^6/ml for 30 min at 37°C (other dyes such as calcein can be used). After washing, cells were suspended in PBS at 1 x 10^6/ml. PBS (35 μl) and 15 μl (15 μg) of peptide solution was added into a protein-coated well, and then 5 x 10^4 cells (50 μl) labeled with dye were added. After vortexing gently, the plate was incubated at room temperature for 25 min in the dark. Each well was gently washed with 100 μl PBS twice. Remaining cells were solubilized in 100 μl/well of PBS containing 0.2% NP40 and fluorescence was measured by a microplate reader.

The data is shown as percent of added cells that remained adherent. Control values "0" and "1000" represent the adhesion observed when no CD66a protein is adherent to the well, or when buffer is added without peptide, respectively. Control values "0.01" represent the adhesion observed in the presence of a blocking CD66 antibody.

Assay for binding of peptides coupled to beads to CHO cells expressing recombinant CD66a. CHO cells transfected with BGPa cDNA were grown and prepared as in Assay #2. To each tube containing 10 μl peptide-bound beads (approximately 300,000 beads) 20 μl of cell suspension was added and mixed gently. The tubes were then incubated for 30 minutes at room temperature.

After incubation, aliquots were taken from each tube and placed on a glass slide. For each sample, data was quantified by viewing five separate fields under a scope at 125x magnification and counting the number of beads associated with single cells or groupings of cells in three size classes.

Example 1 – Effect of peptides on neutrophil activation determined by adhesion to endothelial cells

The CD66a peptides were tested for their ability to alter neutrophil adhesion to human umbilical vein endothelial cells (HUVECs) stimulated for 48 hours with 1000 U/ml gamma-interferon and 50 ng/ml TNF-alpha (Fig. 1). When neutrophils were incubated for 30 min in the presence of media containing 167 μg/ml of each peptide with these HUVECs, and washed as described in the Endothelial Adhesion Assay, three peptides (CD66a peptides CD66a-1, CD66a-2, and CD66a-3) augmented neutrophil adhesion approximately two-fold compared with media (Fig. 1, solid bars). This effect was more prominent in the presence of 10^{-7} M FMLP (hatched bars). In contrast, the other peptides did not alter neutrophil adhesion when compared with incubation in media alone. Similar results were obtained using HUVECs stimulated for 4 hours with 50 μg/ml TNF-alpha (not shown).

The three peptides that specifically induced neutrophil adhesion were further tested for their effects on the adhesion of neutrophils to TNF stimulated HUVECs. Each of the three CD66a peptides, CD66a-1, CD66a-2, and CD66a-3, increased neutrophil adhesion to HUVECs at concentrations as low as 50 μg/ml (approximately 35 μM) in the presence of FMLP (Fig. 2). To confirm that the activity of these peptides was due to the sequence and not simply a charge effect, three scrambled versions were made of each active peptide (Table

II) and tested in the adhesion assay. In contrast to the native peptides, none of the 9 scrambled peptides had activity in the adhesion assay (Fig. 3).

Example 2 – Effect of peptides on neutrophil activation determined by expression of surface CD11b and CD62L

Effect of CD66a peptides on CD11b expression. The effects of the peptides on surface expression of CD11b on neutrophils was next examined. While neutrophil adhesion to HUVECs is dependent on the functional activity of surface CD11/CD18, many adhesive stimuli also upregulate the surface expression of CD11/CD18, and this may play a role in regulating cell adhesion as well (70-72). To determine if an alteration in the surface expression of CD11/CD18 could contribute to the effect of the CD66a peptides on neutrophil adhesion, CD11b expression was analyzed by flow cytometry. Since CD11 and CD18 are translocated to the cell surface only when they are complexed with each other, the use of a directly labeled CD11b mAb was used to demonstrate upregulation of CD18 as well as CD11b. When neutrophils were incubated with HBSS for 15 min at 37°C and then reacted with a PE-labeled CD11b mAb, CD11b expression was readily detected by flow cytometry (MCF = 584) (Fig. 4, top panel). As expected, when neutrophils were incubated with FMLP (10^{-7} M) for 15 min, CD11b expression was increased (MCF = 709) (second panel). When neutrophils were incubated with 167 μg/ml of the CD66a peptide CD66a-1 (MCF = 704) (third panel), the CD66a peptide CD66a-2 (MCF = 713) (fourth panel), or the CD66a peptide CD66a-3 (MCF = 714) (fifth panel), CD11b expression also increased, similar to that seen with incubation with 10^{-7} M FMLP. In contrast, incubation with the scrambled CD66a peptide CD66a-1-S1 resulted in similar CD11b expression as incubation with HBSS (MCF = 581) (bottom panel), as did the other eight scrambled peptides (not shown).

Effect of CD66a peptides on CD62L expression. The effects of the peptides on surface expression of CD62L on neutrophils was next examined. L-selectin, recognized by CD62L mAbs, also plays a role in neutrophil adhesion to endothelial cells, and its expression is altered by stimulation (70, 72). To determine if the surface expression of CD62L could be altered by CD66a peptides, CD62L expression was analyzed by flow cytometry. When

neutrophils were incubated with HBSS for 5 min at 37°C, and then reacted with a PE-labeled CD62L mAb, CD62L expression was readily detected by flow cytometry (MCF = 548) (Fig. 4, top panel). When neutrophils were incubated with 10^{-7} M FMLP, CD62L expression decreased as expected (MCF = 256) (second panel). Similarly, when neutrophils were incubated with the CD66a peptide CD66a-1, (MCF = 230) (third panel), the CD66a peptide CD66a-2 (MCF = 243)(fourth panel), or the CD66a peptide CD66a-3 (MCF = 229) (fifth panel), CD62L expression also decreased. Incubation with the scrambled CD66a peptide CD66a-1-S1 did not alter CD62L expression (MCF = 546) (bottom panel). Similarly, none of the other eight scrambled peptides altered CD62L expression (not shown).

As described above, three other peptides from the N-domains of CD66a, b, c, d, and e, but no other N-domain peptides, were also found to activate neutrophil adhesion to HUVECs (Figs. 5-7).

<u>Example 3 - Modulation of binding of CHO cells expressing recombinant CD66 family members to recombinant CD66 family member proteins in solid phase binding assay #1</u>

Homotypic and heterotypic adhesion was assayed using two different techniques. In Assay #1 the adhesion of CHO cells expressing recombinant CD66a to recombinant CD66a-Fc bound to anti-Fc immobilized to a microtiter well was quantitated in the presence and absence of peptides. Four peptides were found to block CD66a-CD66a adhesion in this assay: CD66a-17; CD66a-18; CD66a-19; and CD66a-24 (Fig. 8).

In Assay #2 the adhesion of CHO cells expressing the appropriate CD66 family member to the desired recombinant CD66 family member immobilized to a microtiter well was quantitated in the presence and absence of peptides (Figs. 9-12).

<u>Example 4 – Binding of microbeads coupled to peptide CD66a-24 to CHO transfectants expressing CD66a</u>

One application of these peptides is their use to target binding of larger structures to specific cells/tissues. The complexing of one or more of the

described peptides to a larger entity should result in binding of the complex to cells expressing the appropriate ligands (for example, CD66a in tumors or CD66a in growing endothelial cells involved in angiogenesis).

CD66a-24 and CD66a-1 peptides were coupled to microbeads and the microbeads were incubated with a suspension of CHO cells expressing CD66a at room temperature for 30 min. The binding of the microbeads to the CHO cells was quantified by counting the number of beads associated with single cells or groups of cells in three cell-group size classes and are reported as the number of microbeads bound to each size group of cells (Fig. 13A). Fig. 13B shows the number of beads associated with single cells, which are reported as the average number of microbeads bound to each single cell. The lack of binding of CD66a-1 coupled beads serves as a negative control for this experiment but does not imply that a different coupling technique would not result in binding.

Example 5 -- Effects of peptides on T-cell activation

Cytotoxic lymphocytes are felt to play a key role in the immune response to malignant transformation. T-cells play an important role in the immune system, and a number of cell-surface molecules have been found to regulate T-cell activation (88, 90, 91, 92). Thus, we tested the effects of CD66 peptides on T-cell activation as determined by proliferation following stimulation by anti-CD3.

Blood lymphocytes were stimulated by anti-CD3 in vitro in the presence of the indicated peptides and proliferation was determined by radioactive nucleotide incorporation. The data are reported as cpm +/- SD. Biological activity was detected in this assay for peptides: CD66a-24 and CD66e-29 (Fig. 14).

Discussion

Peptides were synthesized from regions of CD66 family members that we predict may be exposed on the surface of the molecule. Three of the peptides were found to have activity in an assay examining stimulated neutrophil adhesion to HUVECs. These same three peptides also stimulated

upregulation of CD11b/CD18 and down regulation of CD62L on the neutrophil surface. Scrambled versions of these peptides had no biological activity in either assay, suggesting that the specific amino acid sequence is critical for activity. Thus, the data suggest that peptide motifs from at least three regions of the N-terminal domain of CD66a are involved in the interaction of CD66a with other ligands and can initiate signal transduction in neutrophils. Three other peptides from CD66 family members also stimulated neutrophils.

Several other studies have proposed structural motifs of CD66a family proteins (16, 21, 73).

All neutrophil activating peptides identified in this study are derived from the N-terminal domains of CD66a or CD66e. Studies of transfectants and recombinant proteins have suggested that the N-terminal domain is critical for the homotypic and heterotypic adhesion activity of CD66a (12, 21, 23, 25, 32).. Studies using domain specific mAbs have also suggested that the N-domains of CD66 family members are important in homotypic adhesion (21, 24).. However, studies have also suggested that the A1, B1, or A2 domains may also be important in homotypic adhesion, and may interact with the N-domain (12, 19, 20, 22, 23).

Although carbohydrates on CD66 family members may play important roles, the protein backbone itself appears to have important activity in this and other studies. For example, bacterial fusion proteins free of carbohydrates containing the N or A3B3 domains of CD66e can block CD66e homotypic adhesion, demonstrating that protein-protein interaction is involved in CD66e homotypic adhesion (23). Deglycosylated forms of CD66b and CD66c retain heterotypic adhesion activity (31), further demonstrating that carbohydrates are not necessary for their adhesion functions. In addition, both recombinant N-terminal domains of CD66a and CD66e expressed in Escherichia coli bind Opa proteins with the same specificities as native CD66 molecules, and deglycosylated forms of CD66e bind bacterial Opa proteins (50).

Site directed mutagenesis studies of the related proteins C-CAM-105 and CEA (CD66e) have identified regions important for certain functional activities. For example, the integrity of Arg-98 in the consensus ATPase domain (GPAYSGRET) of C-CAM-105 is essential for homotypic aggregation

(58). This arginine is highly conserved in Ig domains, being important in forming a salt bridge with a highly conserved aspartate within the same domain (16). In our model the consensus ATPase domain is present in the sequence of peptide CD66a-5. However, peptide CD66a-5 had no activity in our assay.

The finding that these short peptides can stimulate neutrophils, as can CD66a mAbs (26-28, 67, 74, 75) suggests that they have significant affinity for a surface structure, possibly native CD66a. If so, whether the activity derives from binding native CD66a and transducing a signal directly, or by another mechanism will require further study. The ability of the synthetic peptides described here to activate neutrophils could be mediated by alterations in CD66a dimerization, possibly by disrupting a preexisting association of CD66a with other CD66 members (including CD66a itself in the form of dimers or oligomers already present on the cell surface) or by stimulating dimerization. It has been suggested that CD66a (76) and CD66e (77) exist on the cell surface as dimers. Dimerization of CD66a could potentially occur via interactions between the extracellular domains of CD66a molecules or via other mechanisms. In other receptor systems (e.g. EGF-monomeric, PDGF-dimeric), it is clear that bivalency of ligand is not necessary to induce receptor dimerization (78-81). Finally, the observed functional "stimulation" could reflect either "stimulation" per se or possibly release from a baseline inhibition.

The mechanisms by which CD66 family members transmit signals (e.g. activation in neutrophils, immune suppression of T-lymphocytes, or growth regulating signals in epithelial cells and carcinomas) are unclear. CD66a is phosphorylated in neutrophils and colon cancer cells (4, 59-61), and associated protein kinase and phosphatase activity may be involved (59, 62). At least eight isoforms of CD66a derived from differential splicing have been described (3, 12, 13, 25). These isoforms contain one N-domain, either three, two, or no Ig C2-like domains, and either a short or a long cytoplasmic tail. Only those isoforms with a long cytoplasmic tail can be phosphorylated on tyrosine, and only the isoform with four Ig domains and a long cytoplasmic tail (the ony isoform detected in neutrophils) have been implicated in signaling. The cytoplasmic domain of neutrophil CD66a contains an immune tyrosine inhibitory motif (ITIM), as well as a motif similar to ITAM (immune tyrosine

activating motif) (3, 59). Phosphorylation of ITAMs and ITIMs leads to binding of protein tyrosine kinases and protein tyrosine phosphatases, respectively, which leads to modification of signal transduction (62, 63). Calmodulin has also been found to bind to the cytoplasmic domain of CD66a, causing an inhibition of homotypic self-association of CD66a in a dot-blot assay (82). CD66a has also recently been shown to dimerize in solution, and calcium-activated calmodulin caused dissociation of CD66a dimers in vitro; suggesting that CD66a dimerization is regulated by calmodulin and intracellular calcium (76). It has been suggested that CD66a dimerization could also be influenced by phosphorylation; CD66a is phosphorylated on Thr-453 in the calmodulin binding site by protein kinase C (3). Clearly, dimerization of CD66a could affect binding of other signal regulating molecules.

CD66 family members appear to be involved in a wide variety of important biological processes, and their differential expression provides the possibility for diverse interactions. For example, CD66a, CD66b, CD66c, and CD66d, but not CD66e, are expressed on neutrophils; CD66e is expressed on many tumor cells but not leukocytes; CD66b is expressed on neutrophils but not epithelial cells; CD66c is expressed on both neutrophils and epithelial cells (reviewed in (1) and (13)). While CD66a was originally described in biliary canaliculi, it has since been found in carcinomas as well as normal tissues, including: sebaceous glands (83, 84), neutrophils, placenta, stomach, breast, pancreas, thyroid, prostate, lung, kidney, uterus, and colon (reviewed in (1) and (25)). The surface expression of these molecules in other cells may also be regulated; for example, CD66a expression is induced on HUVECs following treatment with gamma-IFN (10). In addition, surface expression of CD66 family members may be regulated by other stimuli and this may modify the signal transduction capabilities of cell surface CD66 molecules. Finally, studies have shown that certain bacteria bind to some CD66 family members on neutrophils (45-50, 85, 86) and this interaction may also result in signal transduction resulting in modification of neutrophil activity. The major receptor for murine hepatitis virus is a murine CD66a equivalent (51) (52-55) and studies suggest that this virus uses different murine CD66 family members as the major receptor in different tissues (55). A recent consensus was reached that will

rename the CD66 antigens as follows: CD66a antigen, CEACAM-1; CD66b antigen, CEACAM-8; CD66c antigen, CEACAM-6; CD66d antigen, CEACAM-3, CD66e antigen, CEA (14).

CD66 members appear to play an important role in inflammation. Each of the CD66 family members expressed on neutrophils, CD66a, CD66b, CD66c, and CD66d, are capable of transmitting activation signals in neutrophils, and neutrophil CD66a and CD66c appear to be able to present CD15s (a ligand for ELAM-1 or E-selectin) to E-selectin on endothelial cells in a functional way (26). Recent studies have demonstrated the presence of CD66a on T-lymphocytes and a subset of NK cells (CD16-, CD56+) that predominate in decidua (87), and CD66a is upregulated in activated T-cells (87). Finally, CD66e expression by tumor cells is correlated with resistance to NK/LAK cell mediated lysis (88, 89). Thus, these data suggest that soluble CD66 family members could contribute to the immunosuppression often found in patients with cancer.

The biological activity of the peptides identified here suggests that they may have sufficient affinity to make them potential candidates for drug localization to cells expressing the appropriate surface structures.

Table I: CD66a Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66a-1	SMPFNVAEGKEVL		1	Incr PMN Adhesion to HUVECs
CD66a-2	LVHNLPQQLFGYSW		2	Incr PMN Adhesion to HUVECs
CD66a-3	KGERVDGNRQIVGY		3	Incr PMN Adhesion to HUVECs
CD66a-4	VGYAIGTQQATPG		117	
CD66a-5	ATPGPANSGRETIY		118	
CD66a-6	LLIQNVTQNDTGFY	CD66c-6	119	
CD66a-7	VIKSDLVNEEATGQ	CD66c-7 CD66d-7 CD66e-7	4	Incr PMN Adhesion to HUVECs
CD66a-8	EATGQFHVYPELPK	CD66c-8 Contains CD66d-8	120	
CD66a-9	NNSNPVEDKDAVAF	CD66b-9 CD66c-9	121	
CD66a-10	PETQDTTYLWWINN		5	Homolog CD66b-10, CD66c-10
CD66a-11	NNQSLPVSPRLQLS	CD66e-12 CD66e-27	122	
CD66a-12	LQLSNGNRTLTLLS	CD66b-12	6	Homolog CD66c-12
CD66a-13	TLLSVTRNDTGPYE		123	
CD66a-14	IQNPVSANRSDPVT		124	

CD66a-15	SDPVTLNVTYGPDT	CD66b-15 CD66c-15	7	Incr CD66a-CD66a Adhesion (Transfectant Binding Assay #2)
CD66a-16	PSDTYYRPGANLSL		8	Decr CD66a-CD66a Adhesion (Transfectant Binding Assay #2)
CD66a-17	AASNPPAQYSWLIN		9	Decr CD66a-CD66a Adhesion (Transfectant Binding Assays #1 and #2)
CD66a-18	LINGTFQQSTQELF		10	Decr CD66a-CD66a Adhesion (Transfectant Binding Assay #1)
CD66a-19	FIPNITVNNSGSYT	CD66e-21	11	Decr CD66a-CD66a Adhesion (Transfectant Binding Assay #1)
CD66a-20	ANNSVTGCNRTTVK		125	
CD66a-21	TTVKTIIVTELSPV		12	Incr CD66a-CD66a Adhesion (Transfectant Binding Assay #2)
CD66a-22	ELSPVVAKPQIKAS		126	
CD66a-23	SKTTVTGDKDSVNL		13	Incr CD66a-CD66a Adhesion (Transfectant Binding Assay #2)
CD66a-24	TNDTGISIRWFFKN		14	Decr CD66a-CD66a Adhesion (Transfectant Binding Assay #1)
CD66a-25	KNQSLPSSERMKLS		127	

CD66a-26	ERMKLSQGNTTLSI		15	Incr CD66a-CD66a Adhesion (Transfectant Binding Assay #2)
CD66a-27	LSINPVKREDAGTY		128	
CD66a-28	FNPISKNQSDPIM		129	
CD66a-29	ISKNQSDPIMLNVN		16	Homolog CD66e-31
CD66a-5L	GTQQATPGPANSGR	Shift CD66a-5 to left	130	
CD66a-5R	SGRETIYPNASLLI	Shift CD66a-5 to right	131	
CD66a-6L	TIYPNASLLIQNVT	CD66c-6L Shift CD66a-6 to left	17	Incr PMN Adhesion to HUVECs; Decr CD66a-CD66a Adhesion Decr CD66b-CD66c Adhesion (Transfectant Binding Assay #2)

Table II: Scrambled Versions of CD66a Peptides

Peptide Name	Peptide Sequence	SEQ ID NO:	Function
CD66a-1	SMPFNVAEGKEVL	1	Incr PMN Adhesion to HUVECs
CD66a-1-S1	LEFKVEMAPSNVG	132	
CD66a-1-S2	PNVELEFGMKAVS	133	
CD66a-1-S3	ENMPLSAFEVVKG	134	
CD66a-2	LVHNLPQQLFGYSW	2	Incr PMN Adhesion to HUVECs
CD66a-2-S1	QNLLSHLGFVWPQY	135	
CD66a-2-S2	HVQSFLLWPNLYQG	136	
CD66a-2-S3	SVLPLGQWHQYNFL	137	
CD66a-3	KGERVDGNRQIVGY	3	Incr PMN Adhesion to HUVECs
CD66a-3-S1	VENQGVGGKRIRDY	138	
CD66a-3-S2	GRYDQNKVIEVRGG	139	
CD66a-3-S3	GIVEYKGVDQRNRG	140	

Table III: CD66b Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66b-1	AVPSNAAEGKEVL		18	Homolog CD66a-1
CD66b-2	LVHNLPQDPRGYNW		19	Homolog CD66a-2, CD66e-2
CD66b-3	KGETVDANRRIIGY		20	Homolog CD66a-3
CD66b-4	IGYVISNQQITPG		141	
CD66b-5	ITPGPAYSNRETIY		142	
CD66b-6	LLMRNVTKNDTGSY		143	
CD66b-7	VIKLNLMSEEVTGQ		21	Homolog CD66a-7
CD66b-8	EVTGQFSVHPETPK		144	
CD66b-9	NNSNPVEDKDAVAF	CD66a-9 CD66c-9	121	
CD66b-10	PETQNTTYLWWVNG		22	Decr CD66b-CD66c Adhesion (Transfectant Binding Assay #2)
CD66b-11	NGQSLPVSPRLQLS	CD66c-11 CD66e-43	145	
CD66b-12	LQLSNGNRTLTLLS	CD66a-12	6	Homolog CD66c-12
CD66b-13	TLLSVTRNDVGPYE	CD66e-29	146	
CD66b-14	IQNPASANFSDPVT		147	

CD66b-15	SDPVTLNVTYGPDT	CD66a-15 CD66c-15	7	Incr CD66a-CD66a Adhesion
CD66b-16	PSDTYYHAGVNLNL		23	Homolog CD66a-16
CD66b-17	AASNPPSQYSWSVN		24	Homolog CD66a-17, CD66c-17, CD66e-19
CD66b-18	SVNGTFQQYTQKLF		25	Homolog CD66a-18
CD66b-19	IPNITTKNSGSYA		26	Homolog CD66a-19, CD66c-19
CD66b-20	TTNSATGRNRTTVR		148	
CD66b-21	TTVRMITVSDALVQ		27	Homolog CD66a-21
CD66b-5L	SNQQITPGPAYSNR	Shift CD66b-5 to left	149	
CD66b-6L	TIYPNASLLMRNVT	Shift CD66b-6 to left	28	Homolog CD66a-6L

Table IV: CD66c Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66c-1	STPFNVAEGKEVL	CD66d-1 CD66e-1	29	Homolog CD66a-1
CD66c-2	LAHNLPQNRIGYSW		30	Homolog CD66a-2, CD66e-2
CD66c-3	KGERVDGNSLIVGY	CD66d-3	31	Homolog CD66a-3
CD66c-4	VGYVIGTQQATPG	CD66d-4	150	
CD66c-5	ATPGPAYSGRETIY		151	
CD66c-6	LLIQNVTQNDTGFY	CD66a-6	119	
CD66c-7	VIKSDLVNEEATGQ	CD66a-7 CD66d-7 CD66e-7	4	Incr PMN Adhesion to HUVECs
CD66c-8	EATGQFHVYPELPK	CD66a-8 Contains CD66d-8	120	
CD66c-9	NNSNPVEDKDAVAF	CD66a-9 CD66b-9	121	
CD66c-10	PEVQNTTYLWWVNG		32	Decr CD66b-CD66c Adhesion (Transfectant Binding Assay #2)
CD66c-11	NGQSLPVSPRLQLS	CD66b-11 CD66e-43	145	
CD66c-12	LQLSNGNMTLTLLS		33	Decr CD66b-CD66c Adhesion (Transfectant Binding Assay #2)
CD66c-13	TLLSVKRNDAGSYE		152	

CD66c-14	IQNPASANRSDPVT		153	
CD66c-15	SDPVTLNVTYGPDT	CD66a-15 CD66b-15	7	Incr CD66a-CD66a Adhesion (Transfectant Binding Assay #2)
CD66c-16	PSKANYRPGENLNL		34	Homolog CD66a-16
CD66c-17	AASNPPAQYSWFIN		35	Decr CD66b-CD66c Adhesion Decr CD66c-CD66c Adhesion Decr CD66e-CD66e Adhesion (Transfectant Binding Assay #2)
CD66c-18	FINGTFQQSTQELF		36	Homolog CD66a-18
CD66c-19	IPNITVNNSGSYM		37	Decr CD66b-CD66c Adhesion (Transfectant Binding Assay #2)
CD66c-20	AHNSATGLNRTTVT		154	
CD66c-21	TTVTMITVSGSAPV		38	Homolog CD66a-21
CD66c-5L	GTQQATPGPAYSGR	CD66e-5L Shift CD66c-5 to left	155	
CD66c-6L	TIYPNASLLIQNVT	CD66a-6L Shift CD66c-6 to left	17	Incr PMN Adhesion to HUVECs Decr CD66a-CD66a Adhesion Decr CD66b-CD66c Adhesion (Transfectant Binding Assay #2)

Table V: CD66d Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66d-1	STPFNVAEGKEVL	CD66c-1 CD66e-1	29	Homolog CD66a-1
CD66d-2	LVHNLPQHLFGYSW	CD66e-2	39	Decr CD66e-CD66e Adhesion (Transfectant Binding Assay #2) Homolog CD66a-2, CD66e-2
CD66d-3	KGERVDGNSLIVGY	CD66c-3	31	Homolog CD66a-3
CD66d-4	VGYVIGTQQATPG	CD66c-4	150	
CD66d-5	ATPGAAYSGRETIY		156	
CD66d-6	LLIHNVTQNDIGFY		157	
CD66d-7	VIKSDLVNEEATGQ	CD66a-7 CD66c-7 CD66e-7	4	Incr PMN Adhesion to HUVECs
CD66d-8	EATGQFHVY	Part of CD66a-8 and CD66c-8	158	
CD66d-5L	GTQQATPGAAYSGR	Shift CD66d-5 to left	175	
CD66d-6L	TIYTNASLLIQNVT	Shift CD66d-6 to left	40	Homolog CD66a-6L

Table VI: CD66e Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66e-1	STPFNVAEGKEVL	CD66c-1 CD66d-1	29	Homolog CD66a-1
CD66e-2	LVHNLPQHLFGYSW	CD66d-2	39	Decr CD66e-CD66e Adhesion (Transfectant Binding Assay #2)
CD66e-3	KGERVDGNRQIIGY		41	Incr PMN Adhesion to HUVECs
CD66e-4	IGYVIGTQQATPG		159	
CD66e-5	ATPGPAYSGREIIY		160	
CD66e-6	LLIQNIIQNDTGFY		161	
CD66e-7	VIKSDLVNEEATGQ	CD66a-7 CD66c-7 CD66d-7	4	Incr PMN Adhesion to HUVECs
CD66e-8	EATGQFRVYPELPK		162	
CD66e-9	YPELPKPSISSNNS		163	
CD66e-10	NNSKPVEDKDAVAF	CD66e-41	164	
CD66e-11	PETQDATYLWWVNN		42	Homolog CD66b-10, CD66c-10
CD66e-12	NNQSLPVSPRLQLS	CD66a-11 CD66e-27	122	
CD66e-13	LQLSNGNRTLTLFN		43	Homolog CD66c-12
CD66e-14	TLFNVTRNDTASYK		165	

CD66e-15	TQNPVSARRSDSVI		166	
CD66e-16	SDSVILNVLYGPDA		44	Homolog CD66a-15
CD66e-17	NVLYGPDAPTISPL		45	Homolog CD66a-15
CD66e-18	PLNTSYRSGENLNL		46	Homolog CD66a-16
CD66e-19	AASNPPAQYSWFVN		47	Decr CD66b-CD66c Adhesion Decr CD66c-CD66c Adhesion Decr CD66e-CD66e Adhesion (Transfectant Binding Assay #2)
CD66e-20	FVNGTFQQSTQELF		48	Homolog CD66a-18
CD66e-21	FIPNITVNNSGSYT	CD66a-19	11	Decr CD66a-CD66a Adhesion (Transfectant Binding Assay #1)
CD66e-22	AHNSDTGLNRTTVT		167	
CD66e-23	TTVTTITVYAEPPK		49	Homolog CD66a-21
CD66e-24	TVYAEPPKPFITSN		168	
CD66e-25	NNSNPVEDEDAVAL		50	Homolog CD66a-23
CD66e-26	PEIQNTTYLWWVNN		51	Homolog CD66a-24
CD66e-27	NNQSLPVSPRLQLS	CD66a-11 CD66e-12	122	
CD66e-28	LQLSNDNRTLTLLS		52	Homolog CD66a-26
CD66e-29	TLLSVTRNDVGPYE	CD66b-13	146	
CD66e-30	IQNELSVDHSDPVI		169	

CD66e-31	SVDHSDPVILNVLY		53	Decr CD66e-CD66e Adhesion (Transfectant Binding Assay #2)
CD66e-32	SDPVILNVLYGPDD		85	
CD66e-33	NVLYGPDDPTISPS		86	
CD66e-34	PSYTYYRPGVNLSL		87	
CD66e-35	AASNPPAQYSWLID		88	
CD66e-36	LIDGNIQQHTQELF		89	
CD66e-37	ISNITEKNSGLYT		90	
CD66e-38	ANNSASGHSRTTVK		170	
CD66e-39	TTVKTITVSAELPK		91	
CD66e-40	TVSAELPKPSISSN		171	
CD66e-41	NNSKPVEDKDAVAF	CD66e-10	164	

CD66e-42	PEAQNTTYLWWVNG		54	Decr CD66e-CD66e Adhesion (Transfectant Binding Assay #2)
CD66e-43	NGQSLPVSPRLQLS	CD66b-11 CD66c-11	145	
CD66e-44	LQLSNGNRTLTLFN		92	
CD66e-45	TLFNVTRNDARAYV		172	
CD66e-46	IQNSVSANRSDPVT		173	
CD66e-47	SANRSDPVTLDVLY		93	
CD66e-48	SDPVTLDVLYGPDT		94	
CD66e-49	DVLYGPDTPIISPP		95	
CD66e-50	PPDSSYLSGANLNL		96	
CD66e-51	SASNPSPQYSWRIN		97	
CD66e-52	RINGIPQQHTQVLF		98	
CD66e-53	IAKITPNNNGTYA		99	
CD66e-54	VSNLATGRNNSIVK		174	
CD66e-55	NNSIVKSITVSASG		100	

CD66e-5L	GTQQATPGPAYSGR	CD66c-5L Shift CD66e-5 to left	155		S174
CD66e-6L	IIYPNASLLIQNII	Shift CD66e-6 to left	55	Homolog CD66a-6L	S177

Table VII: CD66f(1) Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66f(1)-1	AQPPKVSEGKDVL		56	Homolog CD66a-1
CD66f(1)-2	LVHNLPQNLTGYIW		57	Homolog CD66a-2, CD66e-2
CD66f(1)-3	KGQMRDLYHYITSY		58	Homolog CD66a-3
CD66f(1)-4	TSYVVDGEIIIYG		176	
CD66f(1)-5	IIYGPAYSGRETAY		177	
CD66f(1)-6	LLIQNVTREDAGSY		178	
CD66f(1)-7	IIKGDDGTRGVTGR		59	Homolog CD66a-7
CD66f(1)-8	GVTGRFTFTLHLETPK		179	
CD66f(1)-9	NNLNPRENKDVLNF		180	
CD66f(1)-10	PKSENYTYIWWLNG		60	Homolog CD66b-10, CD66c-10
CD66f(1)-11	NGQSLPVSPRVKRP		181	
CD66f(1)-12	VKRPIENRILILPS		61	Homolog CD66c-12
CD66f(1)-13	ILPSVTRNETGPYQ		182	
CD66f(1)-14	IRDRYGGVRSDPVT		183	
CD66f(1)-15	SDPVTLNVLYGPDL		62	Homolog CD66a-15

CD66f(1)-16	PSFTYYRSGEVLYL		63	Homolog CD66a-16
CD66f(1)-17	ADSNPPAQYSWTIN		64	Homolog CD66a-17
CD66f(1)-18	TINEKFQLPGQKLF		65	Homolog CD66a-18
CD66f(1)-19	IRHITTKHSGLYV		66	Homolog CD66a-19, CD66c-19
CD66f(1)-20	VRNSATGKESSKSM		184	
CD66f(1)-21	SKSMTVEVSEAL		67	Homolog CD66a-21

Table VIII: CD66f(11) Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66f(11)-1	AQPPKVSEGKDVLL		68	Homolog CD66a-1
CD66f(11)-2	LVHNLPQNLPGYFW		69	Homolog CD66a-2, CD66e-2
CD66f(11)-3	KGEMTDLYHYIISY		70	Homolog CD66a-3
CD66f(11)-4	ISYIVDGKIIIYG		185	
CD66f(11)-5	IIYGPAYSGRETVY		186	
CD66f(11)-6	LLIQNVTRKDAGTY		187	
CD66f(11)-7	IIKRGDETREEIRH		71	Homolog CD66a-7
CD66f(11)-8	EEIRHFTFTLYLETPK		188	
CD66f(11)-9	SNLNPREAMEAVRL		189	
CD66f(11)-10	PETLDASYLWWMNG		72	Homolog CD66b-10, CD66c-10
CD66f(11)-11	NGQSLPVTHRLQLS		190	
CD66f(11)-12	LQLSKTNRTLYLFG		73	Homolog CD66c-12
CD66f(11)-13	YLFGVTKYIAGPYE		191	
CD66f(11)-14	IRNPVSASRSDPVT		192	

CD66f(11)-15	SDPVTLNLLPKLPI		74	Homolog CD66a-15
CD66f(11)-16	INNLNPRENKDVLA		75	Homolog CD66a-16
CD66f(11)-17	EPKSENYTYIWWLN		76	Homolog CD66a-17
CD66f(11)-18	WLNGQSLPVSPGVK		77	Homolog CD66a-18
CD66f(11)-19	RPIENRILILPSV		78	Homolog CD66a-19, CD66c-19
CD66f(11)-20	NETGPYQCEIRDRYG		193	
CD66f(11)-21	DRYGGLRSNPVILN		79	Homolog CD66a-21
CD66f(11)-22	RSNPVILNVLYGPD		194	
CD66f(11)-23	DLPRIYPSFTYYRS		80	Homolog CD66a-23
CD66f(11)-24	TESPPAEYFWTIN		81	Homolog CD66a-24
CD66f(11)-25	INGKFQQSGQKLFI		195	
CD66f(11)-26	KLFIPQITRNHSGL		82	Homolog CD66a-26
CD66f(11)-27	SVHNSATGKEISKS		196	
CD66f(11)-28	KEISKSMTVKVSGK		197	
CD66f(11)-29	KWIPASLAVGFYVE		83	Homolog CD66e-31

CD66f(11)-5L	DGKIIIYGPAYSGR	Shift CD66(f)11-5 to left	198	
CD66f(11)-6L	TVYSNASLLIQNVT	Shift CD66(f)11-6 to left	84	Homolog CD66a-6L

Table IX: Peptides Derived from Homology Loops of CD66 Family Members

CD66a Peptide Name	CD66a SEQ ID NO:	CD66b Peptide Name	CD66b SEQ ID NO:	CD66c Peptide Name	CD66c SEQ ID NO:	CD66d Peptide Name	CD66d SEQ ID NO:	CD66e Peptide Name	CD66e SEQ ID NO:	CD66f(1) Peptide Name	CD66f (1) SEQ ID NO:	CD66f(11) Peptide Name	CD66f (11) SEQ ID NO:
CD66a -1	1	CD66b -1	18	CD66c -1	29	CD66d -1	29	CD66e -1	29	CD66f(1) -1	56	CD66f(11) -1	68
CD66a -2	2	CD66b -2	19	CD66c -2	30	CD66d -2	39	CD66e -2	39	CD66f(1) -2	57	CD66f(11) -2	69
CD66a -3	3	CD66b -3	20	CD66c -3	31	CD66d -3	31	CD66e -3	41	CD66f(1) -3	58	CD66f(11) -3	70
CD66a -4	117	CD66b -4	141	CD66c -4	150	CD66d -4	150	CD66e -4	159	CD66f(1) -4	176	CD66f(11) -4	185
CD66a -5	118	CD66b -5	142	CD66c -5	151	CD66d -5	156	CD66e -5	160	CD66f(1) -5	177	CD66f(11) -5	186
CD66a -6	119	CD66b -6	143	CD66c -6	119	CD66d -6	157	CD66e -6	161	CD66f(1) -6	178	CD66f(11) -6	187
CD66a -7	4	CD66b -7.	21	CD66c -7	4	CD66d -7	4	CD66e -7	4	CD66f(1) -7	59	CD66f(11) -7	71
CD66a -8	120	CD66b -8	144	CD66c -8	120	CD66d -8	158	CD66e -8	162	CD66f(1) -8	179	CD66f(11) -8	188
								CD66e -9	163				
CD66a -9	121	CD66b -9	121	CD66c -9	121			CD66e -10	164	CD66f(1) -9	180	CD66f(11) -9	189
CD66a -10	5	CD66b -10	22	CD66c -10	32			CD66e -11	42	CD66f(1) -10	60	CD66f(11) -10	72
CD66a -11	122	CD66b -11	145	CD66c -11	145			CD66e -12	122	CD66f(1) -11	181	CD66f(11) -11	190
CD66a -12	6	CD66b -12	6	CD66c -12	33			CD66e -13	43	CD66f(1) -12	61	CD66f(11) -12	73

CD66a -13	123	CD66b -13	146	CD66c -13	152			CD66e -14	165	CD66f(1) -13	182	CD66f(11) -13	191
CD66a -14	124	CD66b -14	147	CD66c -14	153			CD66e -15	166	CD66f(1) -14	183	CD66f(11) -14	192
CD66a -15	7	CD66b -15	7	CD66c -15	7			CD66e -16	44	CD66f(1) -15	62	CD66f(11) -15	74
								CD66e -17	45				
CD66a -16	8	CD66b -16	23	CD66c -16	34			CD66e -18	46	CD66f(1) -16	63	CD66f(11) -16	75
CD66a -17	9	CD66b -17	24	CD66c -17	35			CD66e -19	47	CD66f(1) -17	64	CD66f(11) -17	76
CD66a -18	10	CD66b -18	25	CD66c -18	36			CD66e -20	48	CD66f(1) -18	65	CD66f(11) -18	77
CD66a -19	11	CD66b -19	26	CD66c -19	37			CD66e -21	11	CD66f(1) -19	66	CD66f(11) -19	78
CD66a -20	125	CD66b -20	148	CD66c -20	154			CD66e -22	167	CD66f(1) -20	184	CD66f(11) -20	193
CD66a -21	12	CD66b -21	27	CD66c -21	38			CD66e -23	49	CD66f(1) -21	67	CD66f(11) -21	79
CD66a -22	126							CD66e -24	168			CD66f(11) -22	194
CD66a -23	13							CD66e -25	50			CD66f(11) -23	80
CD66a -24	14							CD66e -26	51			CD66f(11) -24	81
CD66a -25	127							CD66e -27	122			CD66f(11) -25	195
CD66a -26	15							CD66e -28	52			CD66f(11) -26	82
CD66a -27	128							CD66e -29	146			CD66f(11) -27	196

CD66a -28	129							CD66e -30	169			CD66f(11) -28	197
CD66a -29	16							CD66e -31	53			CD66f(11) -29	83
								CD66e -32	85				
								CD66e -33	86				
								CD66e -34	87				
								CD66e -35	88				
								CD66e -36	89				
								CD66e -37	90				
								CD66e -38	170				
								CD66e -39	91				
								CD66e -40	171				
								CD66e -41	164				
								CD66e -42	54				
								CD66e -43	145				
								CD66e -44	92				
								CD66e -45	172				

								CD66e -46	173				
								CD66e -47	93				
								CD66e -48	94				
								CD66e -49	95				
								CD66e -50	96				
								CD66e -51	97				
								CD66e -52	98				
								CD66e -53	99				
								CD66e -54	174				
								CD66e -55	100				
CD66a -5L	130	CD66b -5L	149	CD66c -5L	155	CD66d -5L	175	CD66e -5L	155			CD66f(11) -5L	198
CD66a -5R	131												
CD66a -6L	17	CD66b -6L	28	CD66c -6L	17	CD66d -6L	40	CD66e -6L	55			CD66f(11) -6L	84

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Sequence Free Text

SEQ ID NOs:1-200	Synthetic Peptides

The complete disclosure of all patents, patent documents, and publications cited herein are incorporated by reference. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

WHAT IS CLAIMED IS:

1. An isolated peptide comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof that modulate the function of at least one CD66 family member and/or at least one ligand thereof.

2. The peptide of claim 1 represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, or 187.

3. The peptide of claim 2 represented by SEQ ID NOs:1, 2, 3, 4, 7-15, 17, 22, 32, 33, 35, 37, 39, 41, 47, 53, or 54.

4. The peptide of claim 1 which is capable of modulating at least one of the following:

activation of neutrophils;

activation or inhibition of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells;

proliferation and/or differentiation of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells;

proliferation and/or differentiation of epithelial cells;

homotypic and/or heterotypic adhesion among CD66 family members; and

adhesion of CD66 family members to other ligands.

5. The peptide of claim 1 which is complexed with a carrier molecule or structure to form a peptide conjugate.

6. The peptide of claim 5 wherein the carrier molecule or structure is selected from the group of microbeads, liposomes, biological carrier molecules, synthetic polymers, biomaterials, and cells.

7. The peptide of claim 6 wherein the peptide conjugate binds to cells expressing a CD66 protein or a CD66 ligand.

8. The peptide of claim 5 wherein the peptide conjugate includes a label.

9. The peptide of claim 1 which is attached to a label.

10. The peptide of claim 9 wherein the label is selected from the group consisting of a fluorescent tag, a radioactive tag, a magnetic resonance tag, an enzymatic tag, and combinations thereof.

11. A method of activating a neutrophil comprising contacting the neutrophil with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1, 2, 3, 4, 17, 41, or analogs thereof.

12. The method of claim 11 wherein the peptide is represented by SEQ ID NOs:1, 2, 3, 4, 17, or 41.

13. The method of claim 11 which is carried out *in vitro*.

14. The method of claim 11 which is carried out *in vivo*.

15. A method of blocking the activation of a neutrophil induced by the method of claim 11, the method comprising contacting the neutrophil when in the presence of at least one of the peptides listed in claim 11 with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:18-21, 28-31, 39, 40, 55-59, 68-71, 84, or analogs thereof.

16. The method of claim 15 wherein the peptide is represented by SEQ ID NOs:18-21, 28-31, 39, 40, 55-59, 68-71, or 84.

17. The method of claim 15 which is carried out *in vitro*.

18. The method of claim 15 which is carried out *in vivo*.

19. A method of modulating the homotypic and/or heterotypic adhesion of CD66 family members or adhesion of a CD66 protein to a CD66 ligand; the method comprising contacting CD66 family members and/or their ligands with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, 54, or analogs thereof.

20. The method of claim 19 wherein the peptide is represented by SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, or 54.

21. The method of claim 19 which is carried out *in vitro*.

22. The method of claim 19 which is carried out *in vivo*.

23. A method of altering the modulation of the homotypic and/or heterotypic adhesion of CD66 family members or adhesion between a CD66 protein and a CD66 ligand induced by the method of claim 19, the method comprising contacting CD66 family members and/or their ligands when in the presence of at least one of the peptides listed in claim 19 with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, 72-100, or analogs thereof.

24. The method of claim 23 wherein the peptide is represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, or 72-100.

25. The method of claim 23 which is carried out *in vitro*.

26. The method of claim 23 which is carried out *in vivo*.

27. A method of modulating immune cell activation, proliferation, and/or differentiation; the method comprising contacting an immune cell with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:14, 53, or analogs thereof.

28. The method of claim 27 wherein the peptide is represented by SEQ ID NOs:14 or 53.

29. The method of claim 27 wherein the immune cell is selected from the group of a T-cell, a B-cell, a LAK cell, an NK cell, a dendritic cell, and combinations thereof.

30. The method of claim 27 which is carried out *in vitro*.

31. The method of claim 27 which is carried out *in vivo*.

32. A method of modulating at least one of the following functions of CD66 family members and/or ligands thereof in cells: activation of neutrophils; activation or inhibition of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells; proliferation and/or differentiation of T-cells, B-cells, LAK cells, NK cells, dendritic cells, or other immune system cells; proliferation and/or differentiation of epithelial cells; homotypic and/or heterotypic adhesion among CD66 family members; and adhesion of CD66 family members to other ligands; the method comprising contacting cells with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

33. A method of delivering a therapeutically active agent to a patient comprising administering at least one peptide conjugate comprising a

peptide and the therapeutically active agent to a patient wherein the peptide comprises an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

34. The method of claim 33 wherein the therapeutically active agent is selected from drugs, DNA sequences, RNA sequences, proteins, lipids, and combinations thereof.

35. The method of claim 33 wherein the therapeutically active agent is an antibacterial agent, antiinflammatory agent, or antineoplastic agent.

36. A method of modifying the metastasis of malignant cells comprising contacting the malignant cells or normal host tissue with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

37. A method of altering bacterial or viral binding to cells or a biomaterial, the method comprising contacting the cells or biomaterial with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

38. A method of altering cell adhesion to a biomaterial, the method comprising contacting the biomaterial with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

39. A method of detecting tumors comprising contacting tumor cells or tumor vasculature with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

40. A method of detecting inflammation comprising contacting inflamed vasculature or leukocytes with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

41. A method of detecting a CD66 protein or a ligand thereof, the method comprising contacting tissue comprising a CD66 protein or a ligand thereof with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

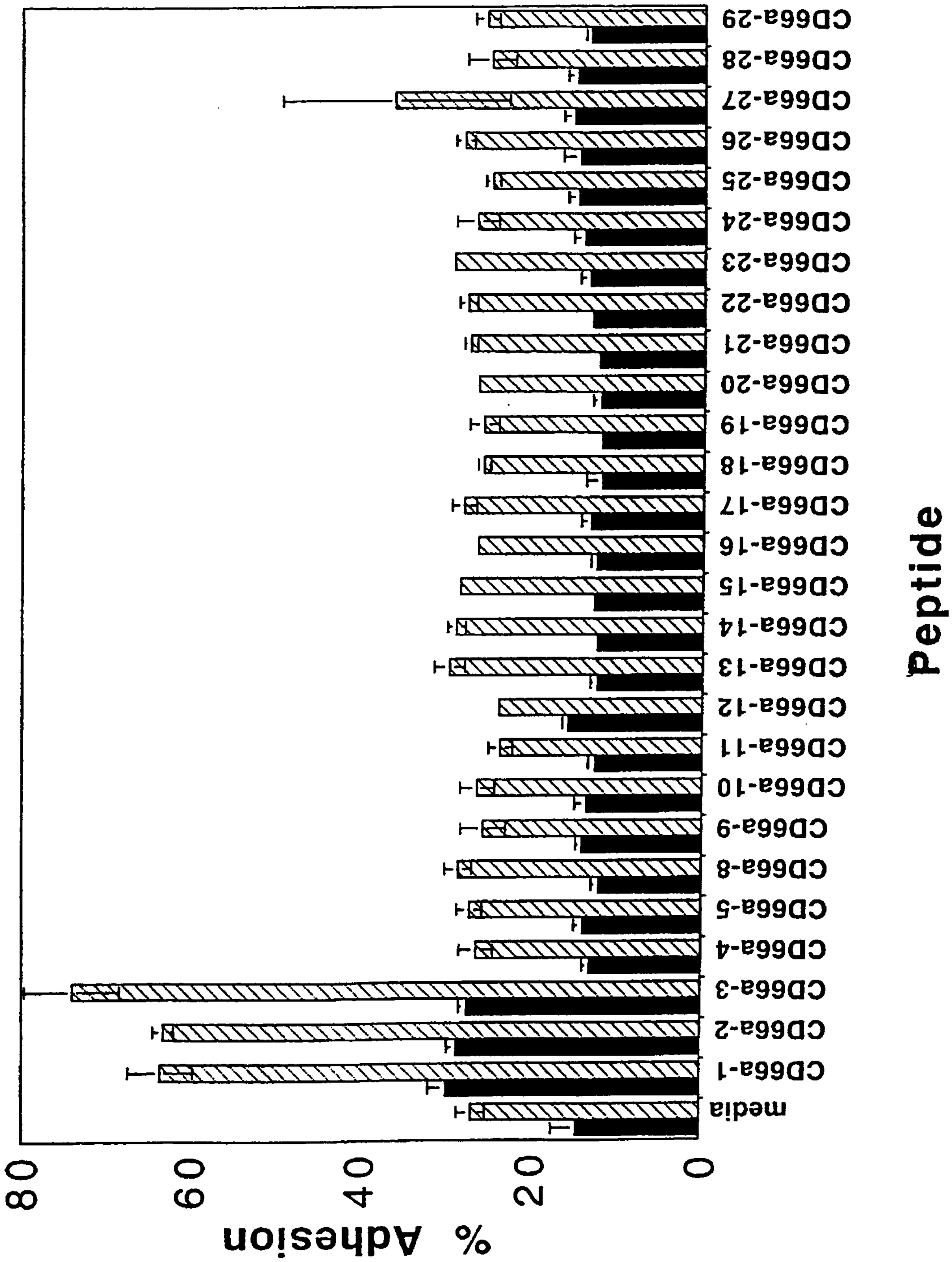
42. A method of altering angiogenesis comprising contacting endothelial cells, tumor cells, or immune cells with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

43. A method of altering an immune response, the method comprising contacting immune system cells with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

44. A method of altering keratinocyte proliferation comprising contacting keratinocytes with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

45. An isolated peptide comprising an amino acid sequence represented by SMPFN (SEQ ID NO:101), PQQLF (SEQ ID NO:102), LPQQL (SEQ ID NO:103), QQLFG (SEQ ID NO:104), NRQIV (SEQ ID NO:105), GNRQI (SEQ ID NO:106), IKSDLVNE (SEQ ID NO:107), AASNPP (SEQ ID NO:108), NTTYLWWVNG (SEQ ID NO:109), YLWWVNG (SEQ ID NO:110), SWLIN (SEQ ID NO:111), SWFIN (SEQ ID NO:112), AQYSWLIN (SEQ ID NO:113), AQYSWFIN (SEQ ID NO:114), SWFVN (SEQ ID NO:115), AQYSWFVN (SEQ ID NO:116),

NRQII (SEQ ID NO:199), GNRQI (SEQ ID NO:200), or analogs thereof.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20
40
60
80
Peptide
media
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CD66a-2
CD66a-3
CD66a-4
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CD66a-8
CD66a-9
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CD66a-11
CD66a-12
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CD66a-29

Figure 1

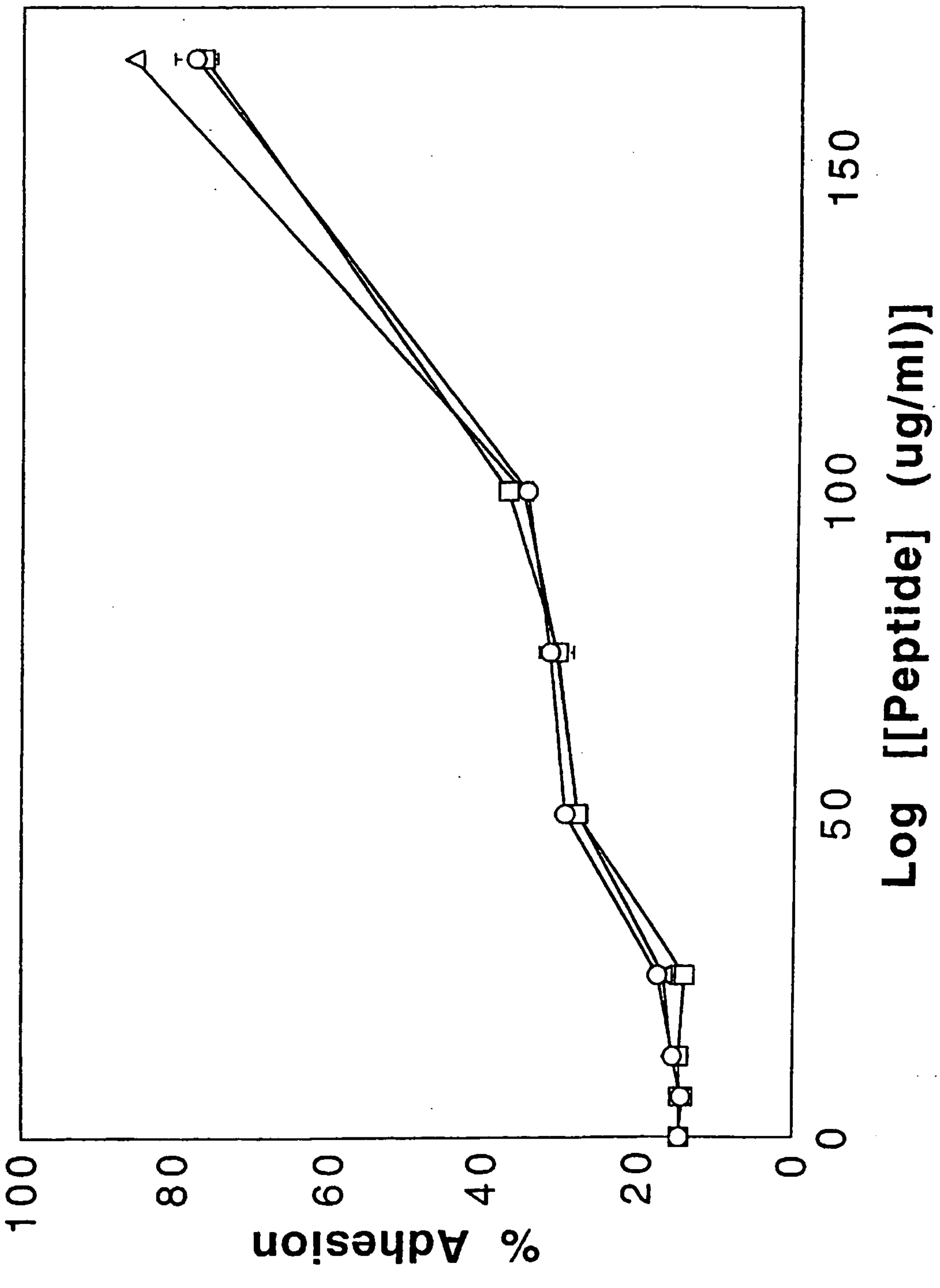
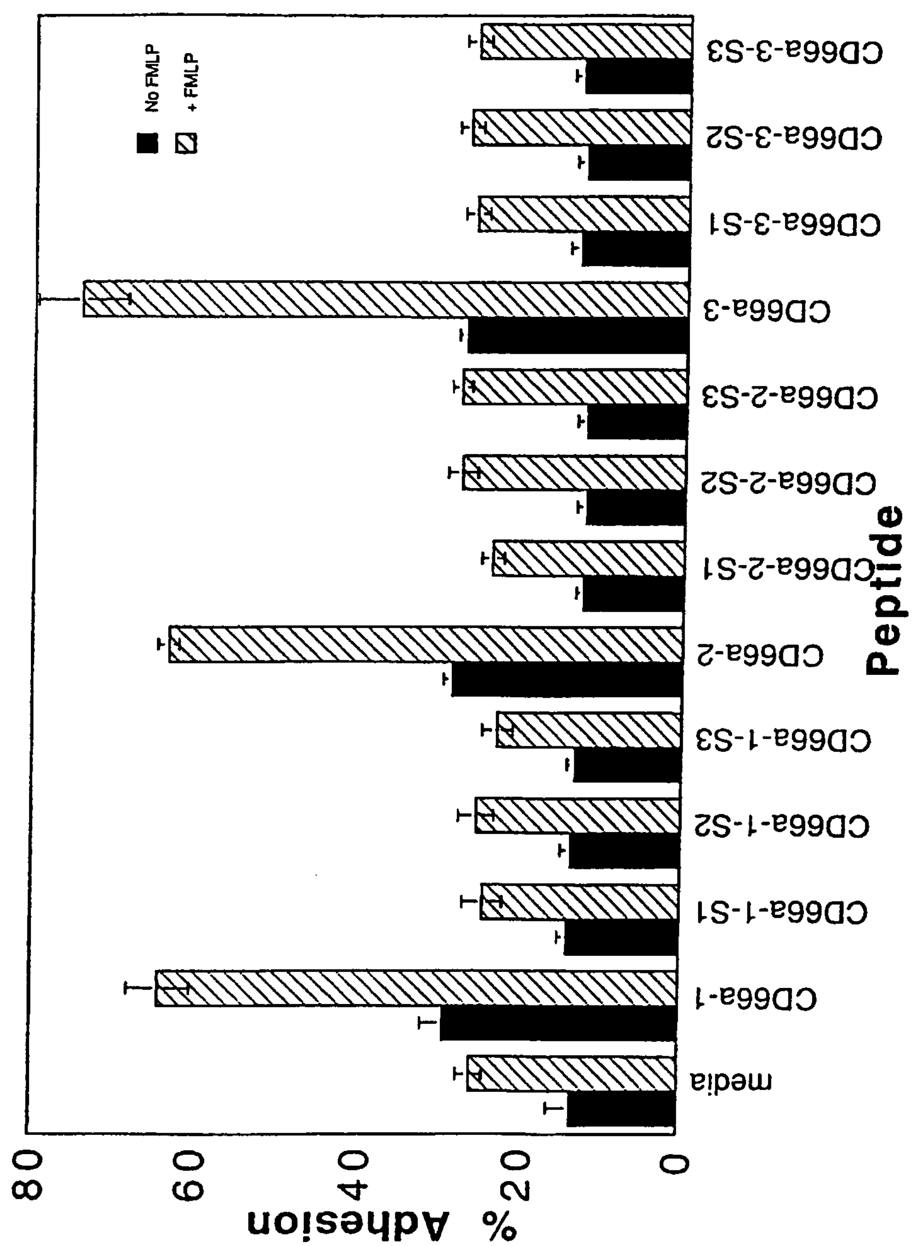


Figure 2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60
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Peptide
media
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CD66a-2-S2
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CD66a-3-S3
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+ FMLP

Figure 3

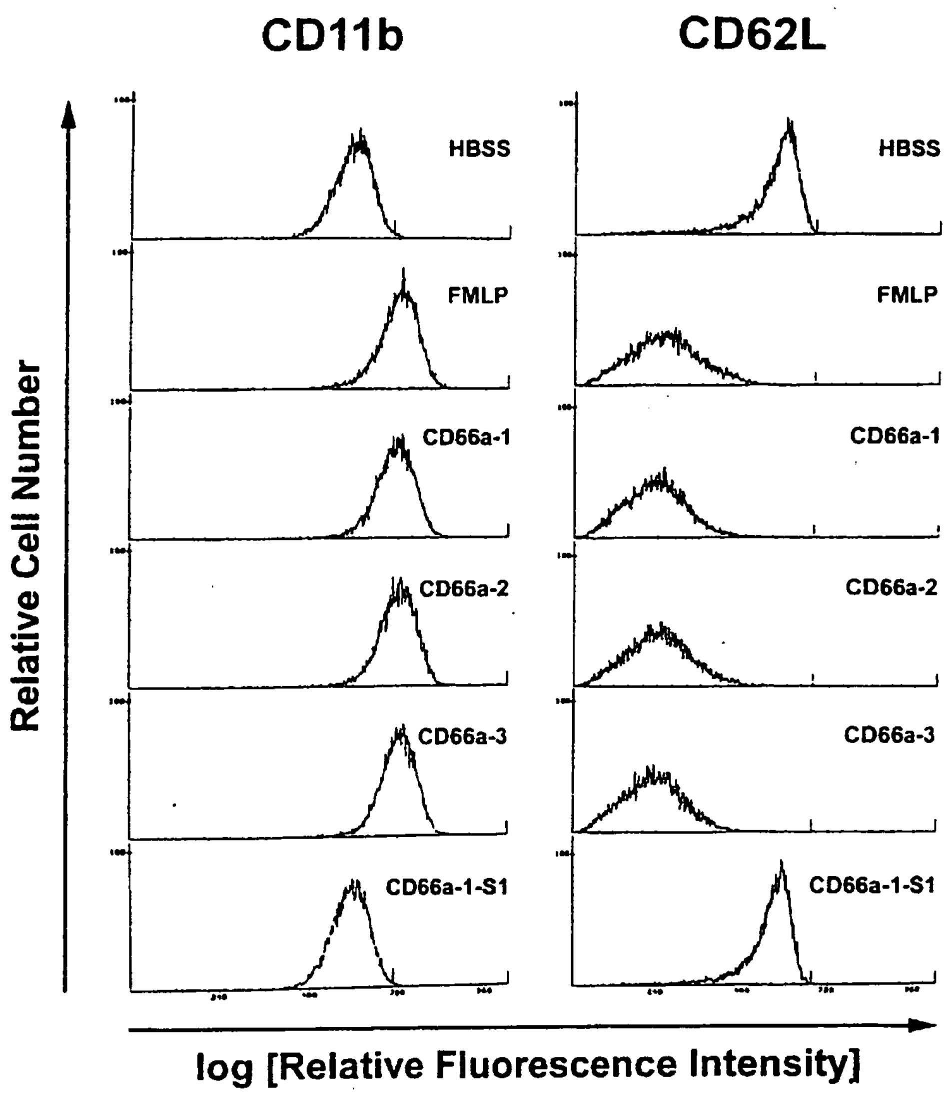

CD11b
CD62L
HBSS
FMLP
CD66a-1
CD66a-2
CD66a-3
CD66a-1-S1
Relative Cell Number
log [Relative Fluorescence Intensity]

Figure 4

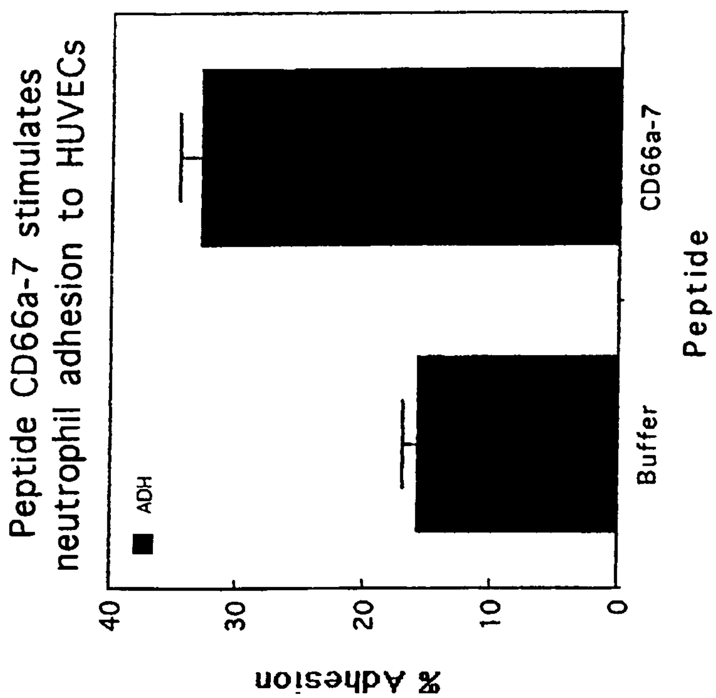

Peptide CD66a-7 stimulates
neutrophil adhesion to HUVECs
ADH
% Adhesion
0
10
20
30
40
Buffer
CD66a-7
Peptide

Figure 5

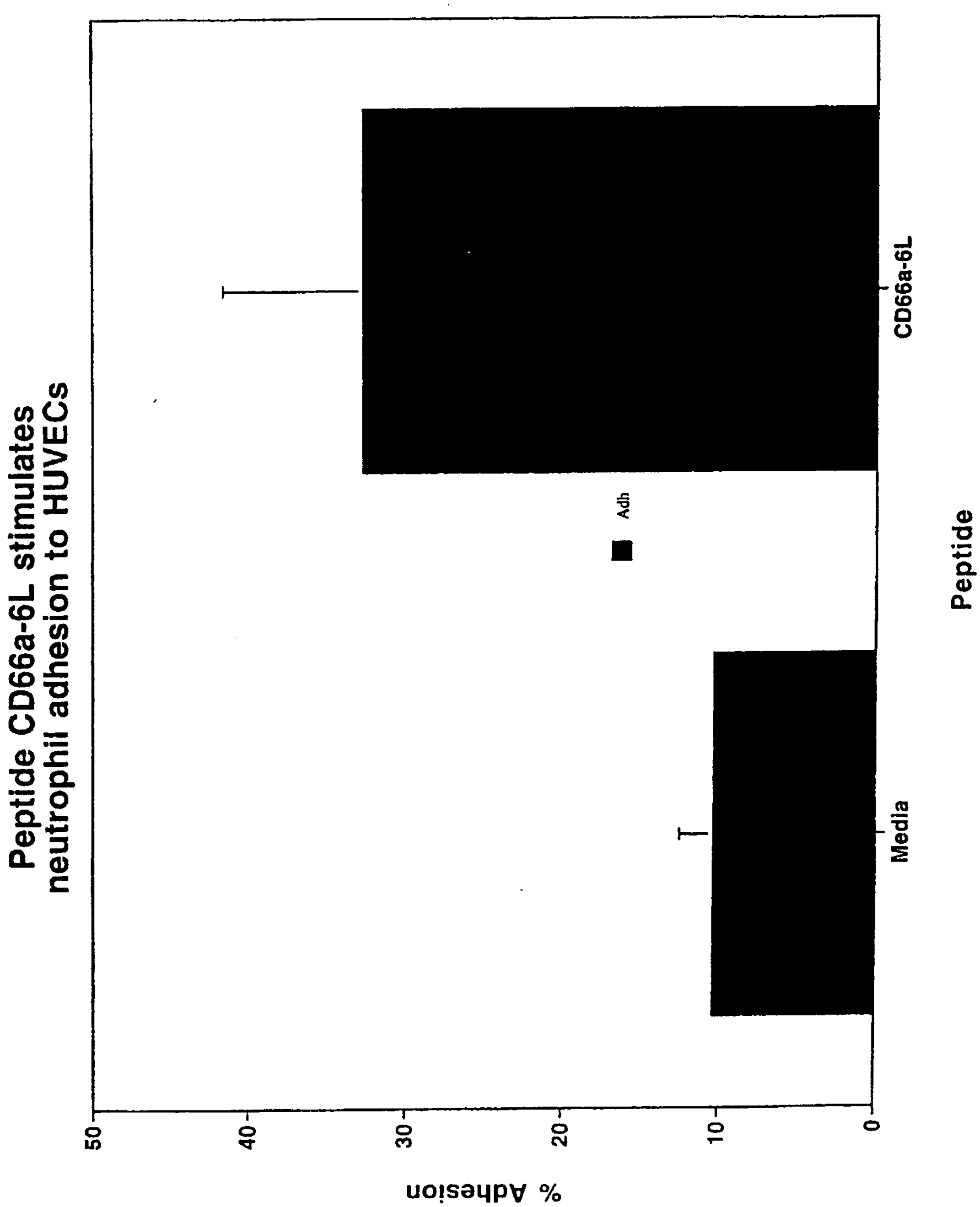
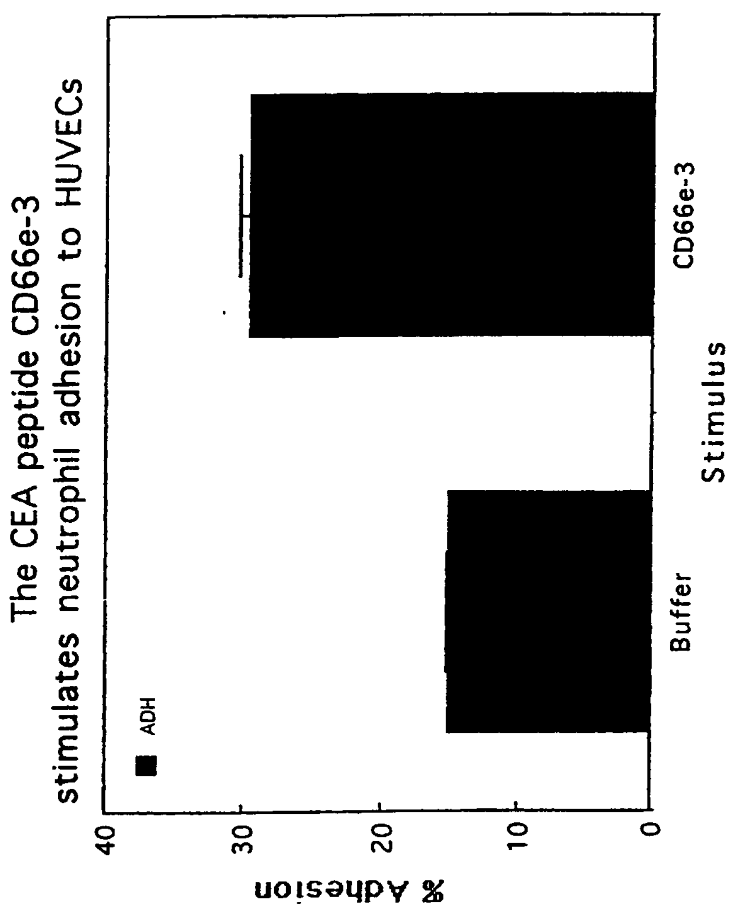

Peptide CD66a-6L stimulates
neutrophil adhesion to HUVECs
% Adhesion
50
40
30
20
10
0
Adh
Media
CD66a-6L
Peptide

Figure 6


The CEA peptide CD66e-3
stimulates neutrophil adhesion to HUVECs
% Adhesion
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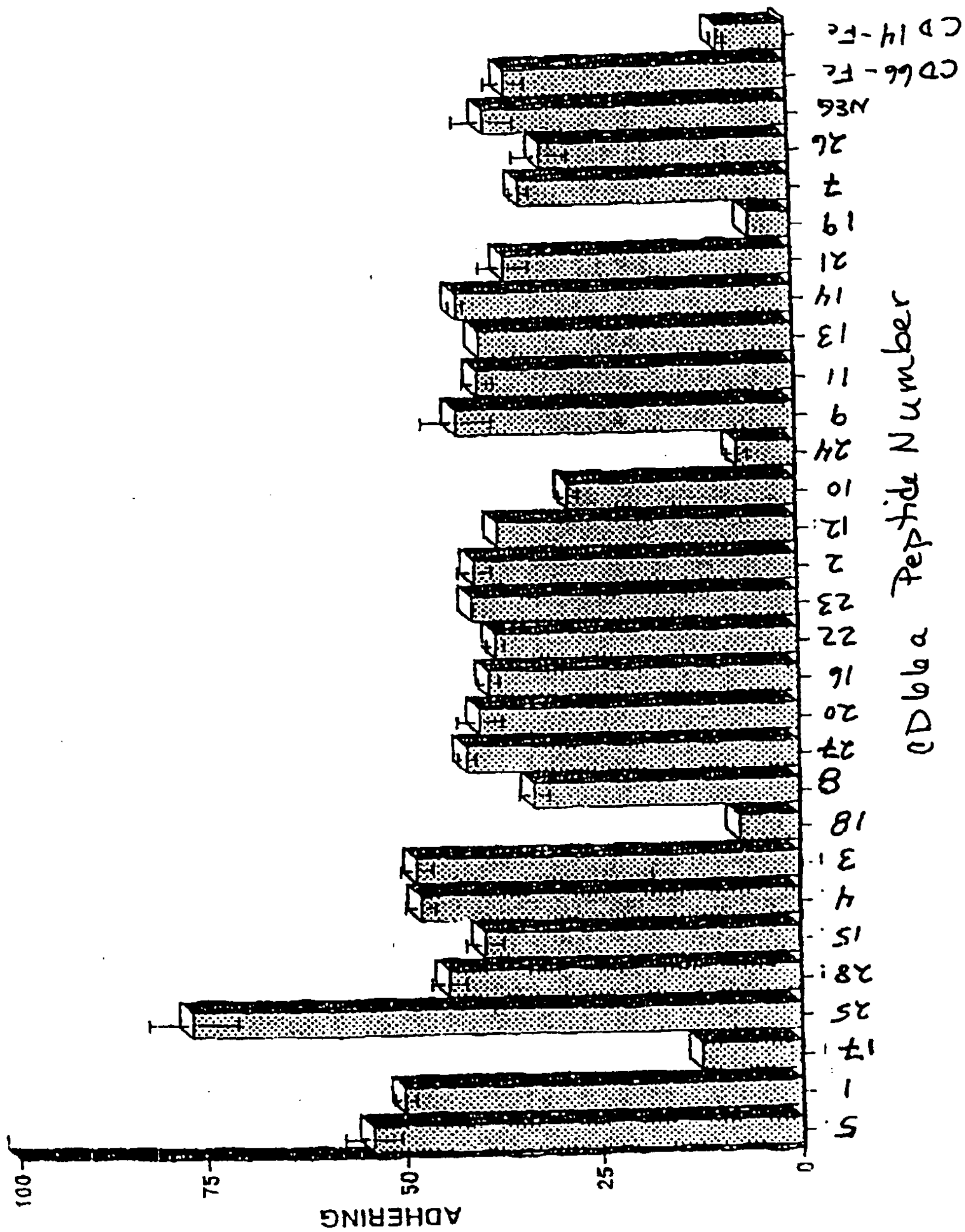
30
20
10
0
ADH
Buffer
CD66e-3
Stimulus

Figure 7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22
23
2
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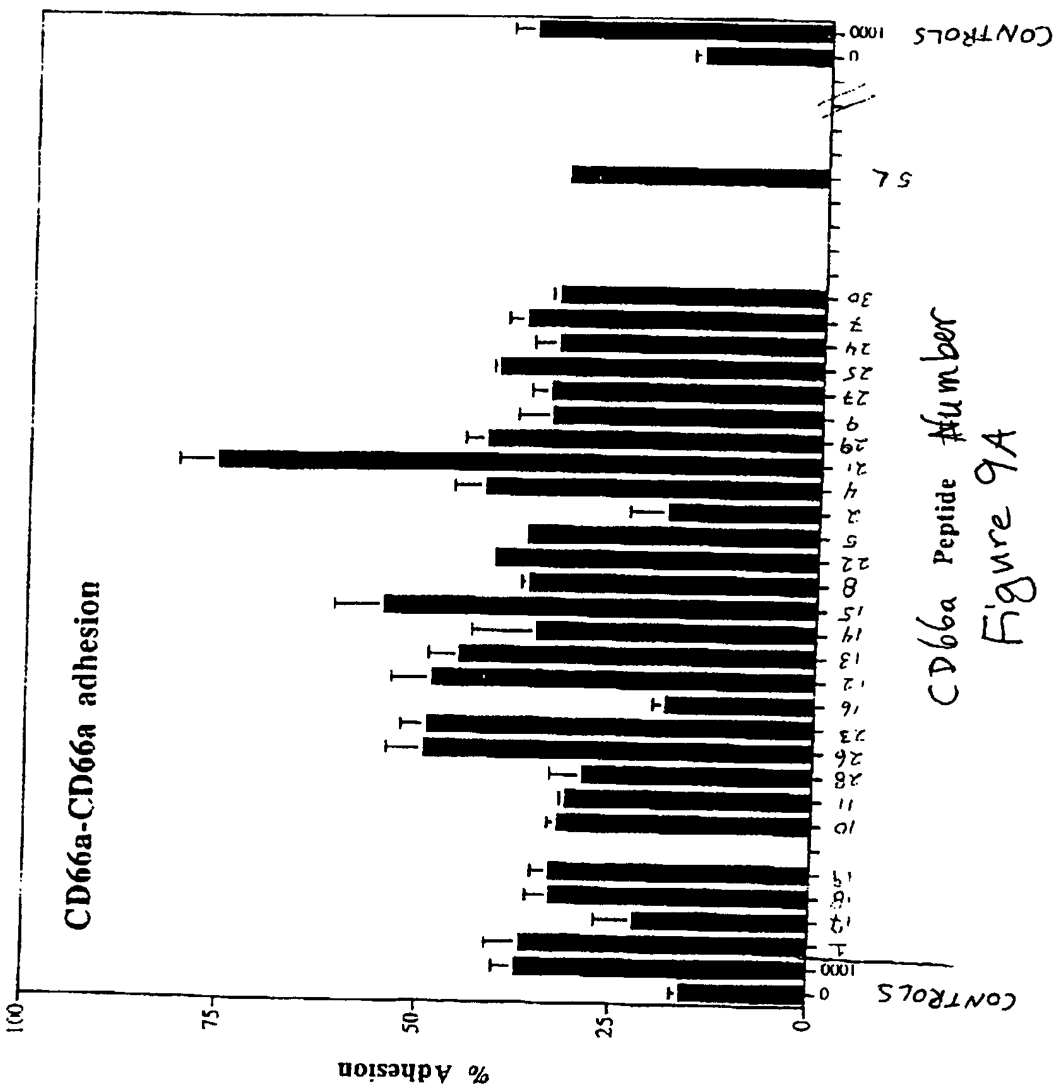
19
7
26
NEG
CD66-Fc
CD14-Fc
CD66a Peptide Number

Figure 8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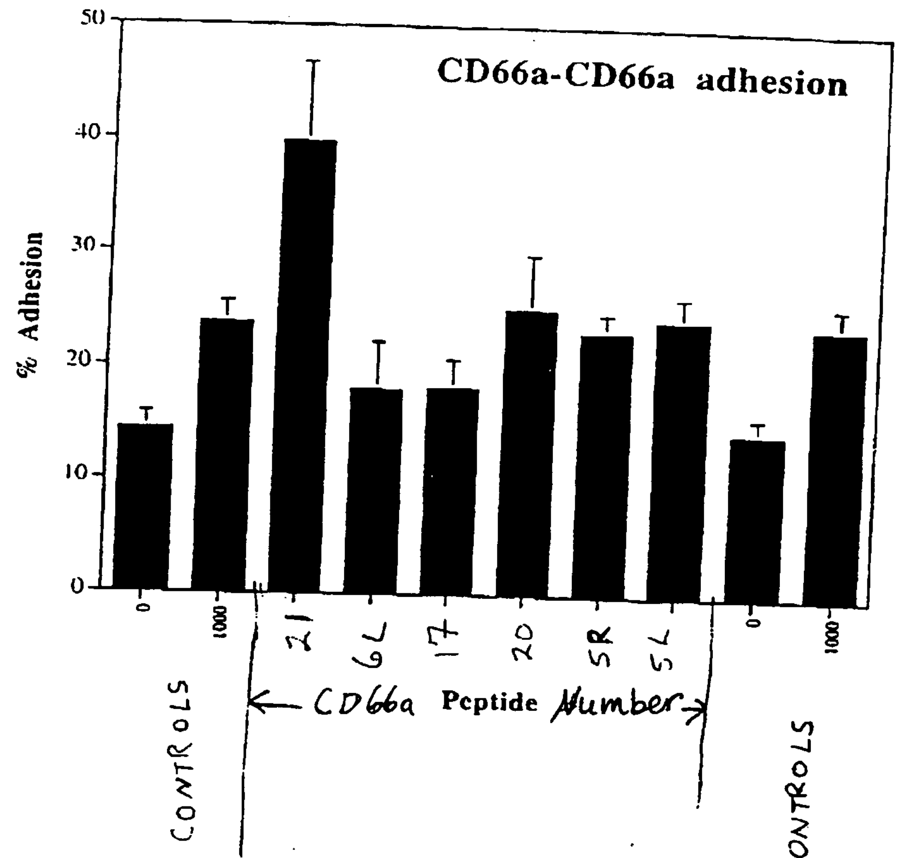
% Adhesion
0
25
50
75
100
CONTROLS
CONTROLS
CD66a Peptide Number

Figure 9A


CD66a-CD66a adhesion
% Adhesion
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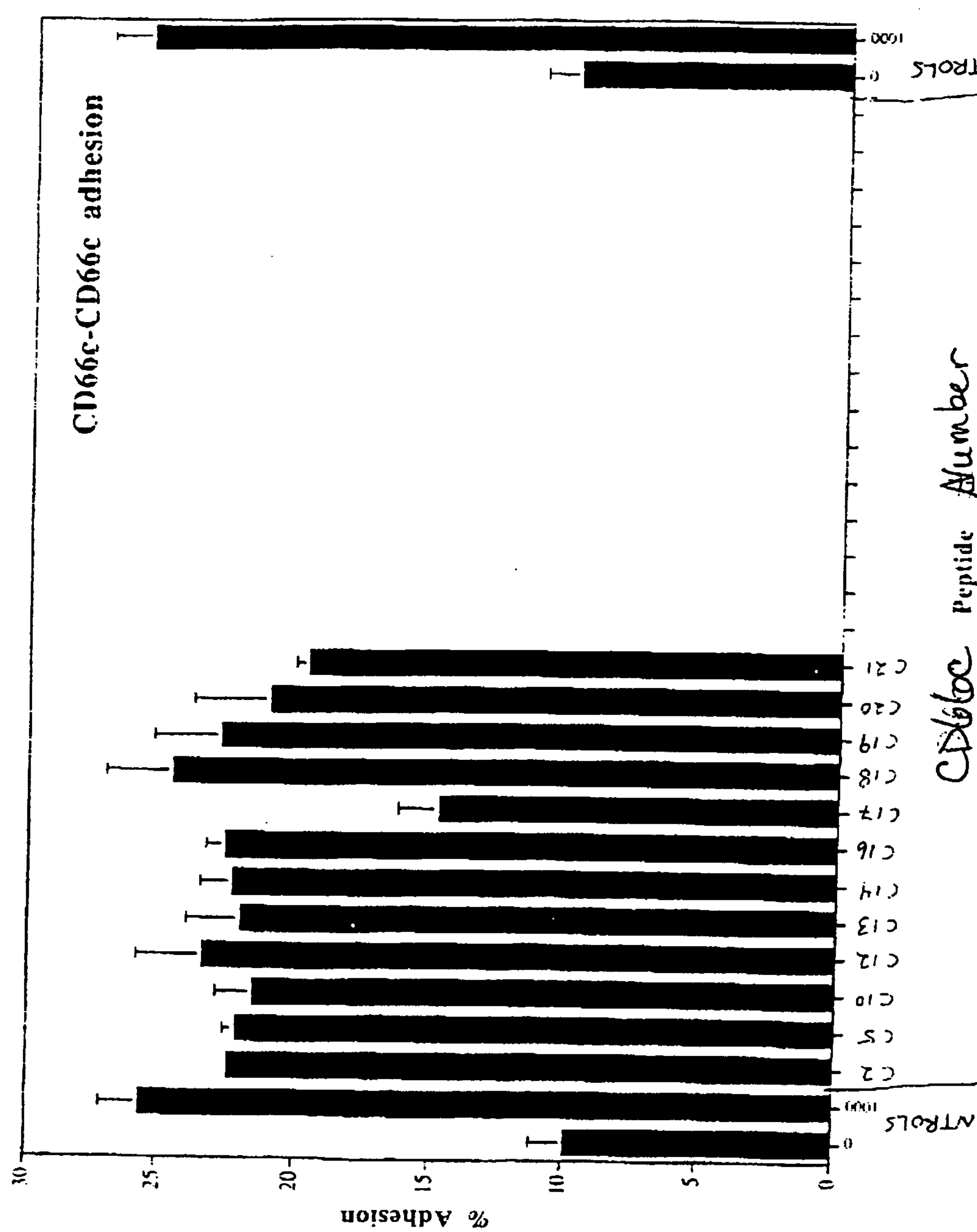
40
30
20
10
0
0
1000
21
6L
17
20
5R
5L
0
1000
CONTROLS
CD66a Peptide Number
CONTROLS

Figure 9B


CD66c-CD66c adhesion
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CD66c Peptide Number
CONTROLS
0
1000
C2
C5
C10
C12
C13
C14
C16
C17
C18
C19
C20
C21
0
5
10
15
20
25
30

Figure 10A

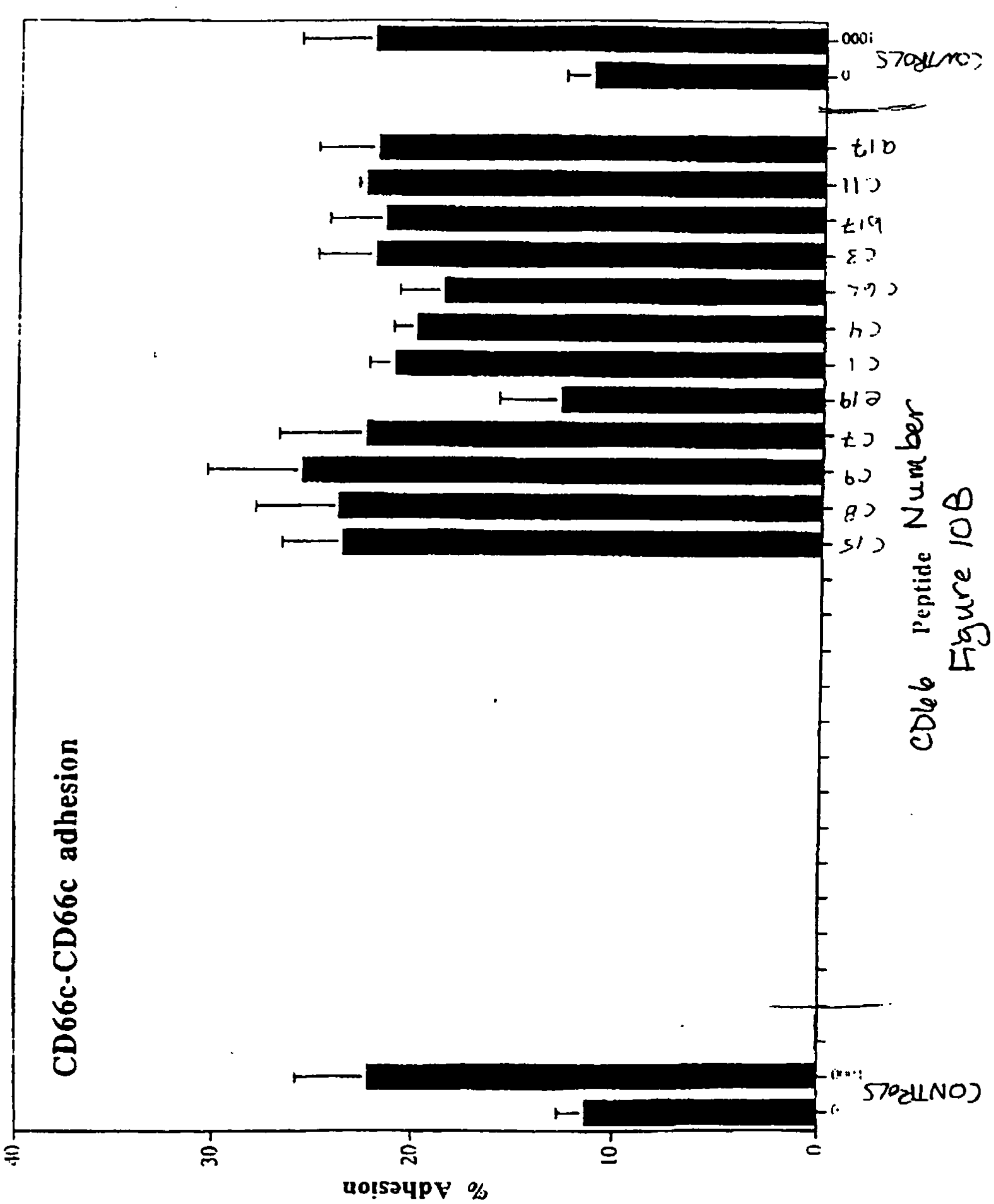

CD66c-CD66c adhesion
% Adhesion
0
10
20
30
40
CONTROLS
c15
c8
c9
c7
e19
c1
c4
c6c
c3
b17
c11
a19
CD66 Peptide Number

Figure 10B

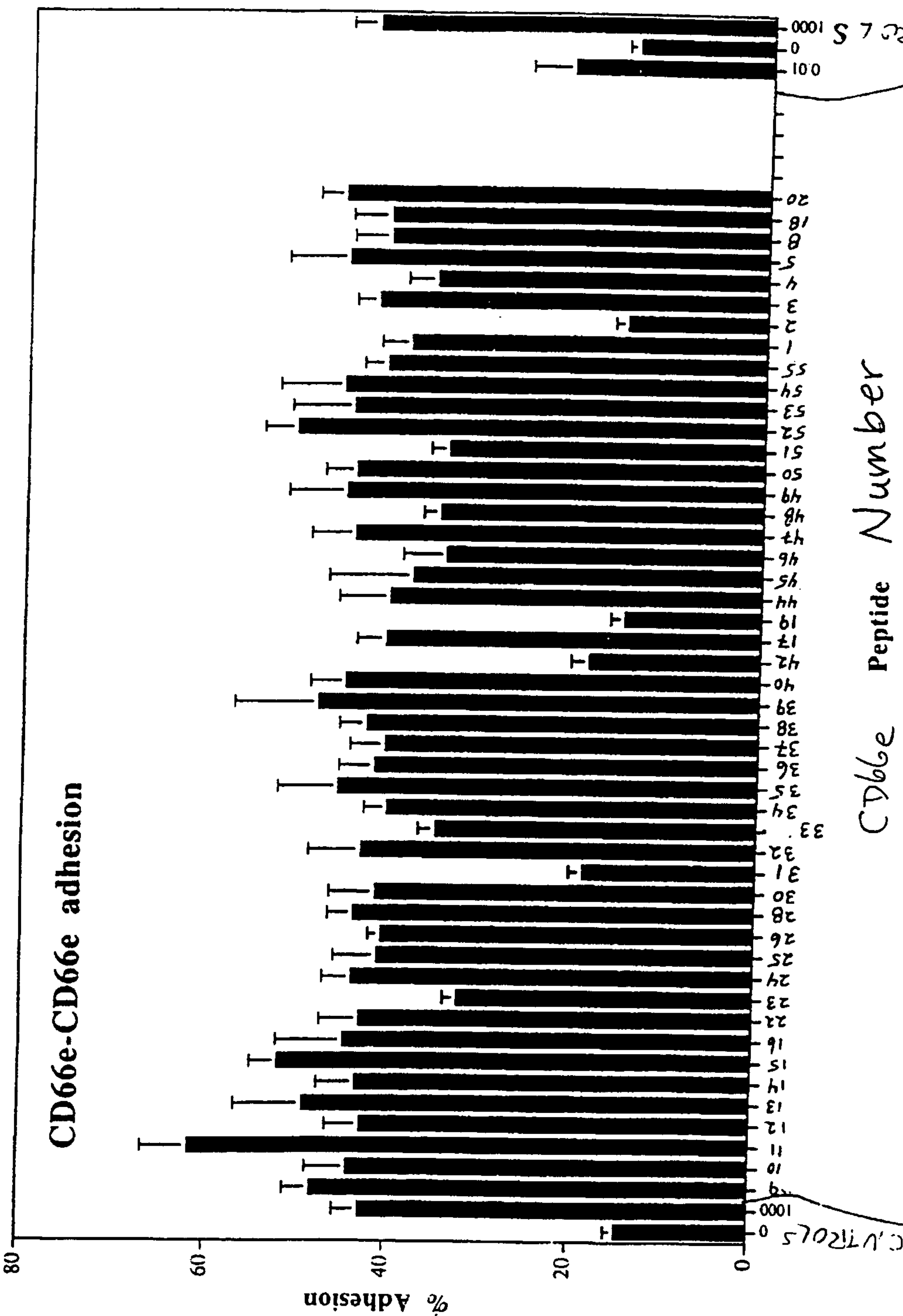

CD66e-CD66e adhesion
% Adhesion
0
20
40
60
80
CONTROLS
CD66e Peptide Number

Figure 11A

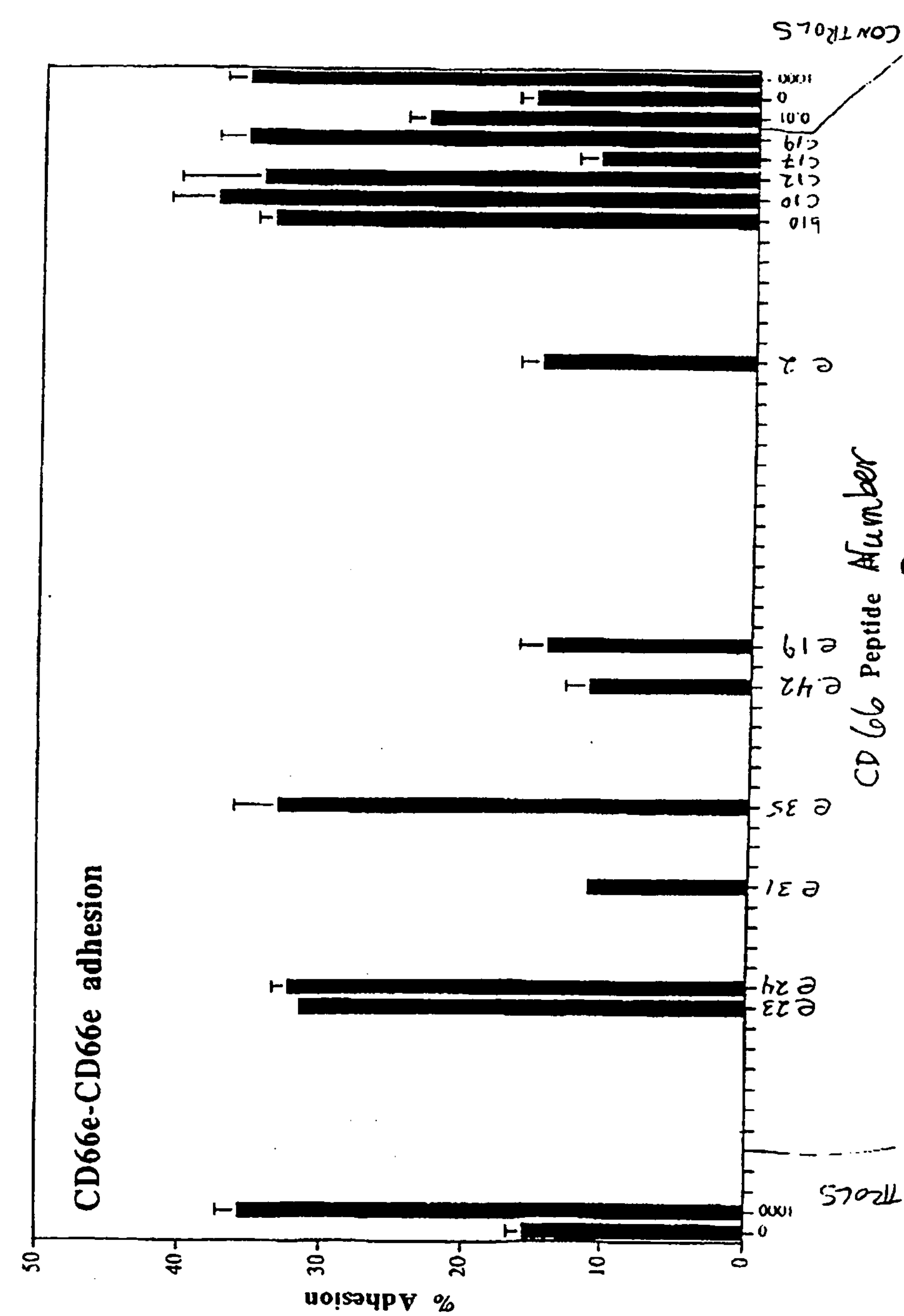

CD66e-CD66e adhesion
% Adhesion
0
10
20
30
40
50
0
1000
CONTROLS
e 23
e 24
e 31
e 35
e 42
e 19
e 2
b10
c10
c12
c17
c19
0.01
0
1000
CONTROLS
CD 66 Peptide Number

Figure 11B

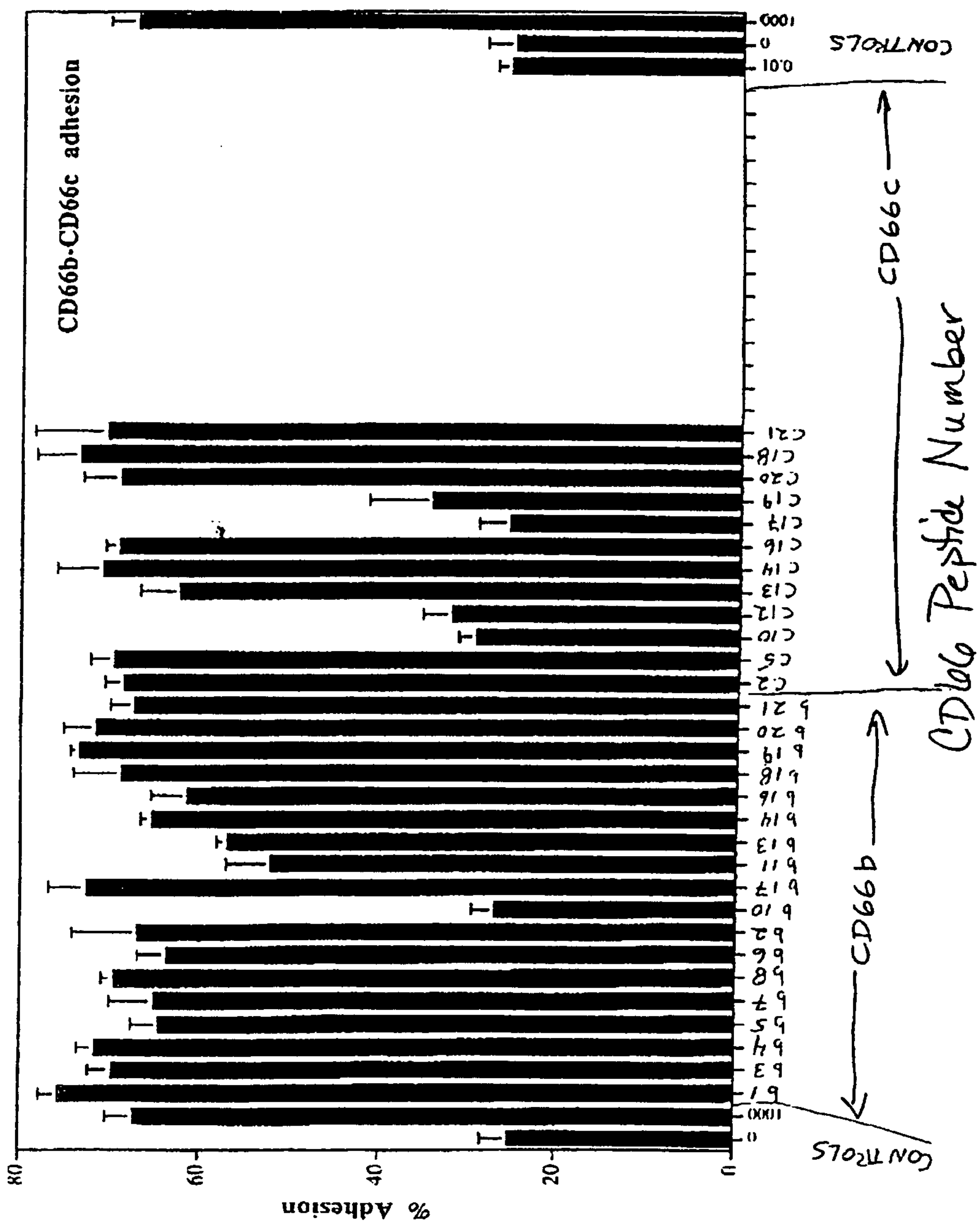
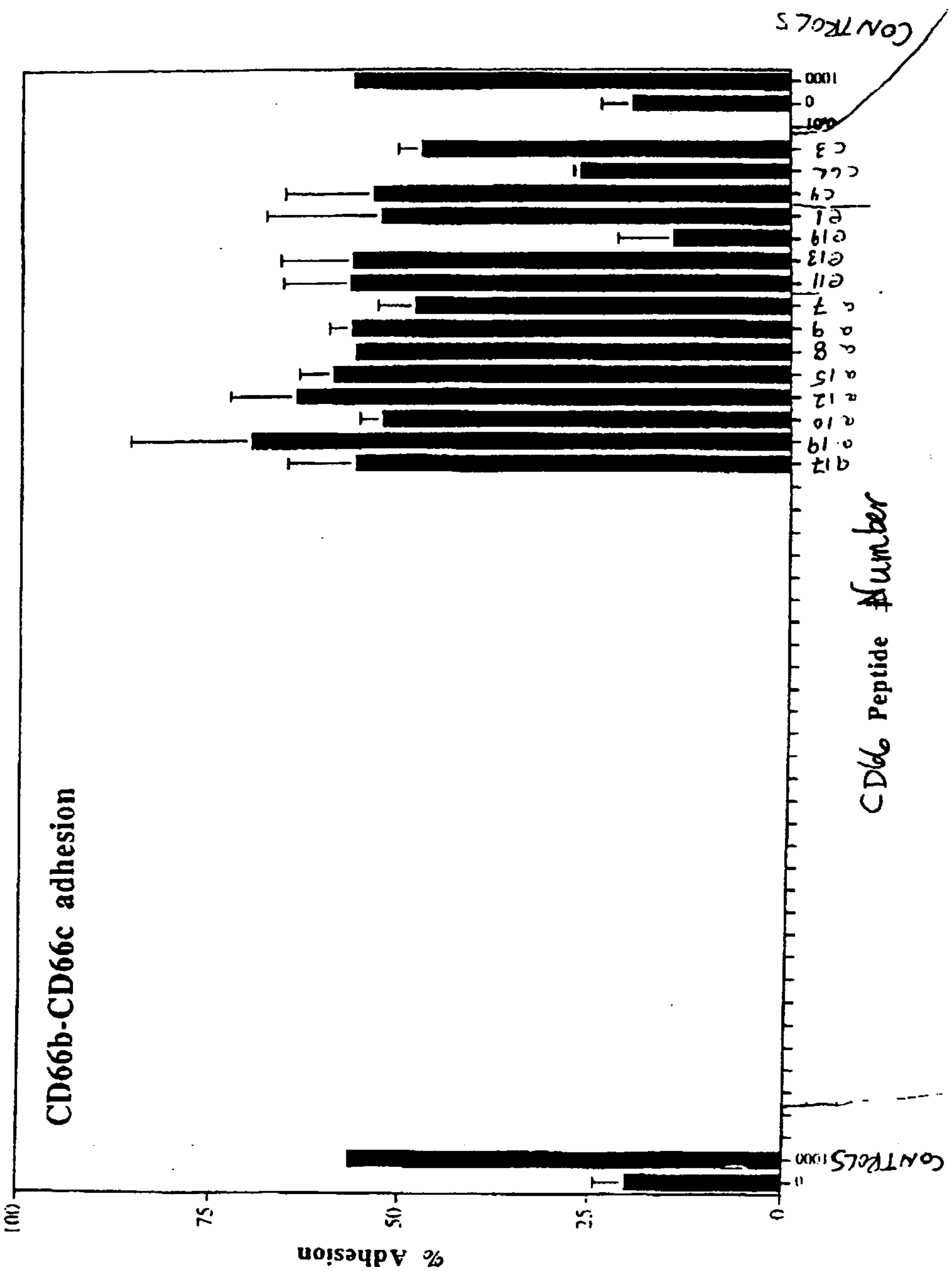

CD66b-CD66c adhesion
% Adhesion
0
20
40
60
80
CONTROLS
CD66b
CD66c
CD66 Peptide Number

Figure 12A

Figure 12B


CD66b-CD66c adhesion
% Adhesion
CD66 Peptide Number
CONTROLS
CONTROLS
0
25
50
75
100

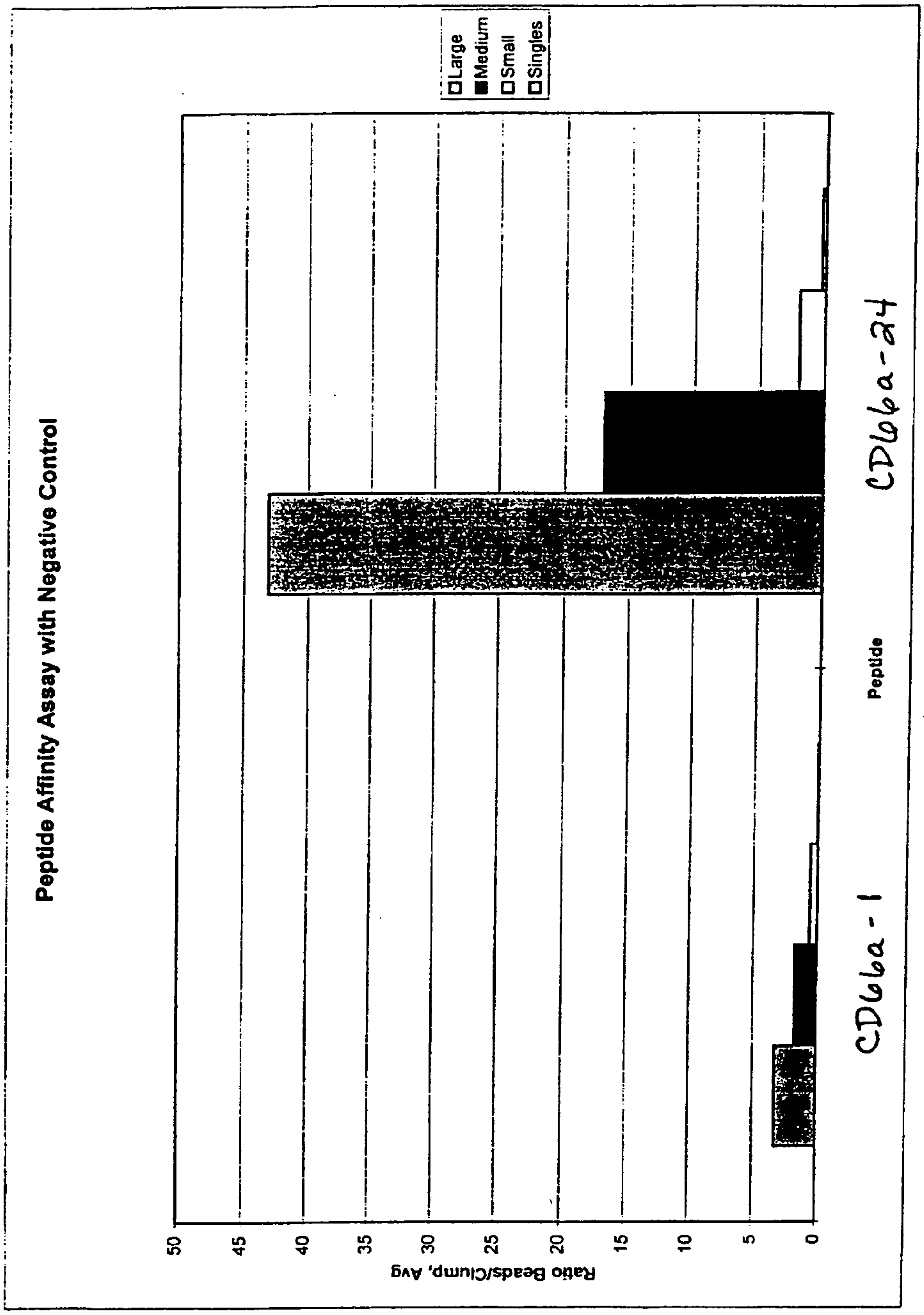

Peptide Affinity Assay with Negative Control
Ratio Beads/Clump, Avg
50
45
40
35
30
25
20
15
10
5
0
Peptide
CD66a-1
CD66a-24
Large
Medium
Small
Singles

Figure 13A

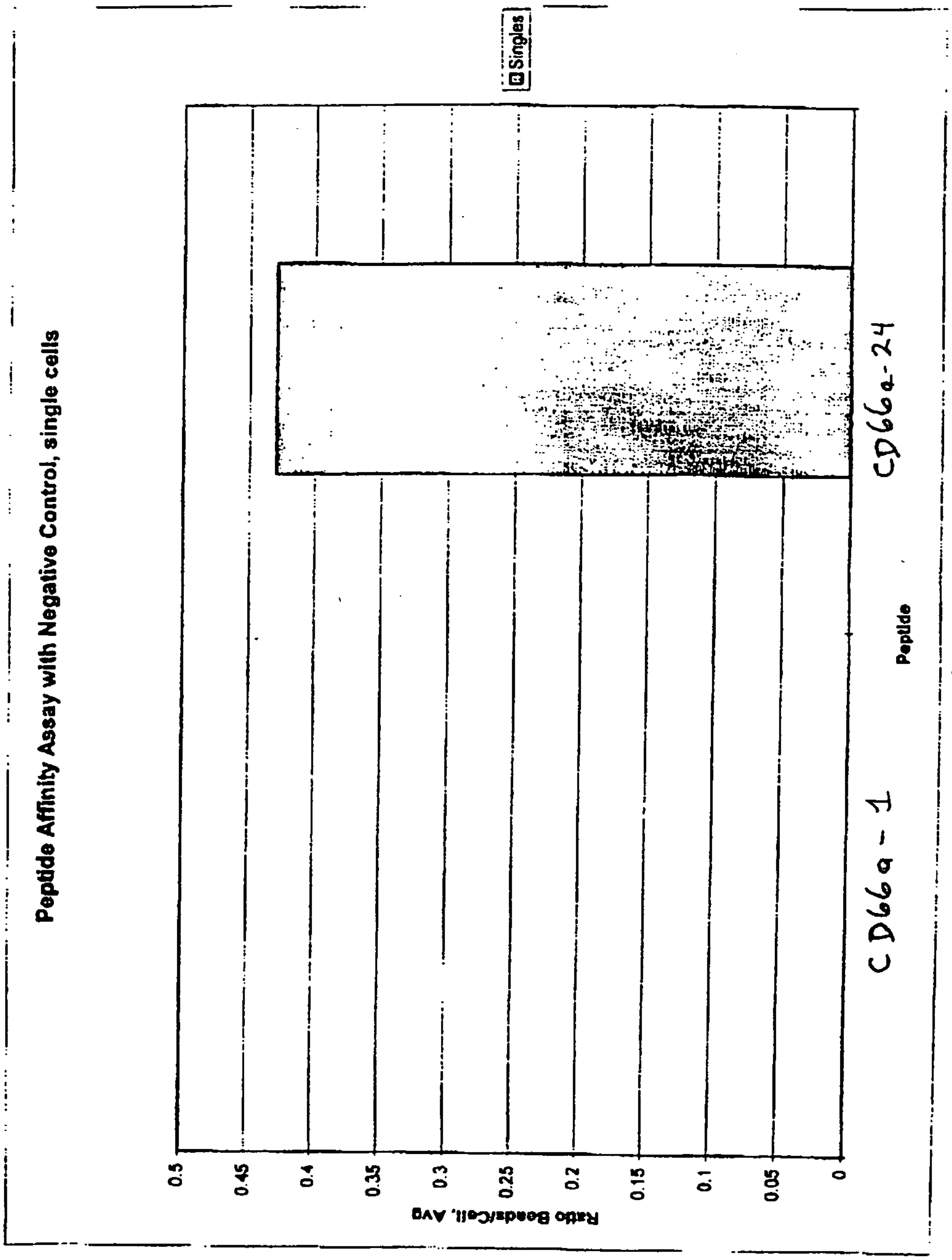

Peptide Affinity Assay with Negative Control, single cells
Singles
Ratio Beads/Cell, Avg
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Peptide
CD66a-1
CD66a-24

Figure 13B

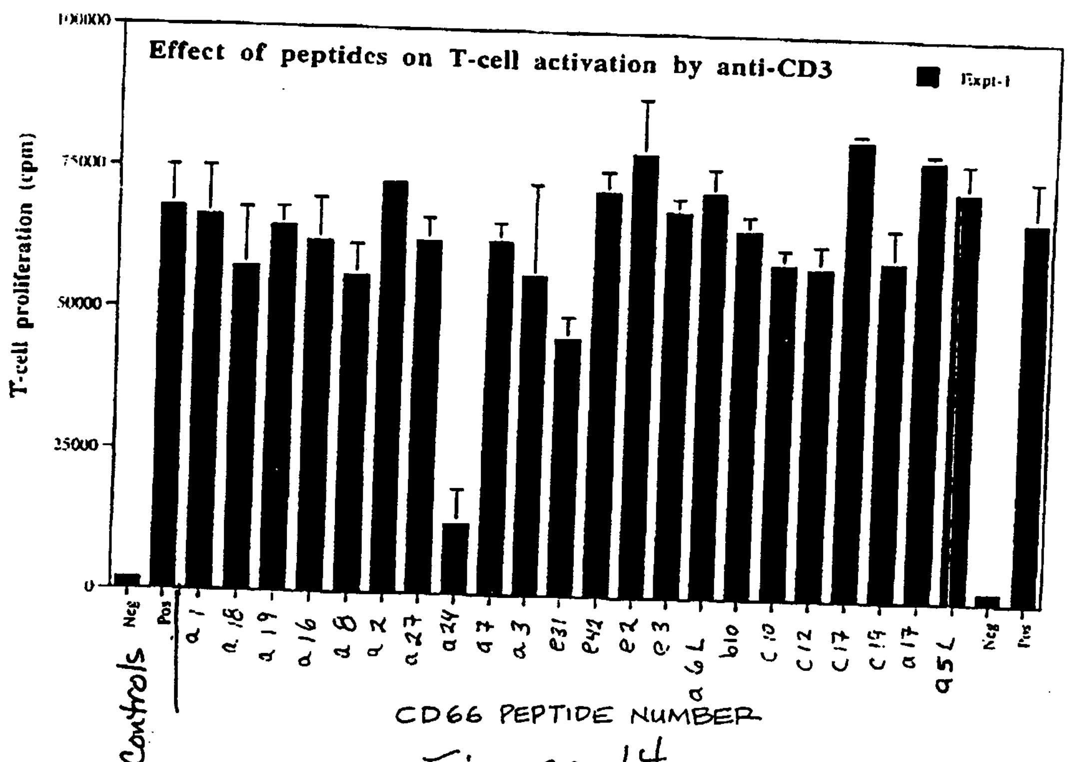

Effect of peptides on T-cell activation by anti-CD3
Expt-1
T-cell proliferation (cpm)
100000
75000
50000
25000
0
Neg
Pos
a 1
a 18
a 19
a 16
a 8
a 2
a 27
a 24
a 7
a 3
e31
e42
e2
e3
a 6L
b10
c 10
c 12
c 17
c 19
a 17
a5L
Neg
Pos
Controls
CD66 PEPTIDE NUMBER

Figure 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/25482

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 38/04, 38/17, 39/00; C07K 7/00, 7/08, 14/435, 17/00
US CL :530/327, 350; 424/184.1, 185.1, 277.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/327, 350; 424/184.1, 185.1, 277.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DIALOG (files 5 and 155) and EAST (files U.S. patents, European abstracts, Japanese abstracts , and Derwent) search terms: CEACAM, BGP, biliary glycoprotein, CD66, CD66a, antigen

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P ------ Y, P	US 5,965,710 A (BODMER et al) 12 October 1999, SEQ ID NO: 32.	1, 4-7 ----------- 8-10
X ---- Y	US 5,571,710 A (BARNETT et al) 05 November 1996, abstract, claims, and columns 26-30.	1, 4-7 -------- 8-10
Y, P ------- A, P	SKUBITZ et al. Stimulation of Neutrophil Adhesion to Endothelial Cells by Synthetic Peptides of CD66a. Molecular Biology of the Cell. November 1999, Vol. 10, supplemental, abstract 452 on page 78A.	1, 4-10 ---------- 2-3

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search 03 JANUARY 2001	Date of mailing of the international search report 25 JAN 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Joyce Bridgers for MARIANNE P. ALLEN JOYCE BRIDGERS PARALEGAL SPECIALIST CHEMICAL MATRIX Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/23482

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	SKUBITZ et al. Synthetic Peptides of CD66a Stimulate Neutrophil Adhesion to Endothelial Cells. Journal of Immunology. 15 April 2000, Vol. 164, No. 8, pages 4257-64, especially abstract and Table I.	1-10
X ---- Y	TEIXEIRA et al. The N-Domain of the Biliary Glycoprotein (BGP) Adhesion Molecule Mediates Homotypic Binding: Domain Interactions and Epitope Analysis of BGPc. Blood. 01 July 1994, Vol. 84, No. 1, pages 211-219, especially abstract and Figure 2.	1, 4-7 -------- 8-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/23482

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10 for SEQ ID NO: 14

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/23482

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-10, drawn to a first set of peptides.
Group II, claims 11-32, drawn to methods of activating or blocking activation of neutrophils.
Group III, claims 19-26 and 32, drawn to methods of modulating and altering modulation of adhesion.
Group IV, claims 27-32, drawn to methods of modulating immune cell activation, proliferation, or differentiation.
Group V, claims 33-35, drawn to methods of delivering therapeutics.
Group VI, claim 36, drawn to a method of modifying metastasis.
Group VII, claim 37, drawn to a method of altering bacterial or viral binding to a biomaterial.
Group VIII, claim 38, drawn to a method of altering cell adhesion to a biomaterial.
Group IX, claim 39, drawn to a method of detecting tumors.
Group X, claim 40, drawn to a method of detecting inflammation.
Group XI, claim 41, drawn to a method of detecting a CD66 protein or ligand.
Group XII, claim 42, drawn to a method of altering angiogenesis.
Group XIII, claim 43, drawn to a method of altering immune response.
Group XIV, claim 44, drawn to a method of altering keratinocyte proliferation..
Group XV, claim 45, drawn to a second set of peptides.

It is noted that claim 32 appears in each of Groups II-IV. This claim is specifically directed to the three different methods of Groups II-IV and will be examined only to the degree that it reflects the elected invention and sequence (see below).

Sequence Election Requirement Applicable to All Groups

In addition, each Group detailed above reads on patentably distinct SEQ ID Numbers. Each sequence is patentably distinct because they are unrelated sequences, and a further restriction is applied to each Group. Applicant must further elect a single SEQ ID Number for an amino acid sequence. Each additional amino acid sequence is considered to be an additional group.

The inventions listed as Groups I-XV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Each of the products of Groups I and XV can be shown to be distinct, each from every other, as their structures differ. In addition, the set of peptides in Groups I and XV appear to be mutually exclusive. Thus, none share a special technical feature. Each of the methods of groups II-XIV do not require each other for their ultimate use and each method has different starting materials, method steps, and/or goals. Thus, they do not share a special technical feature. It is noted that not all of the products are used in each of the methods.

The examiner will rejoin claims directed to the first appearing method using the elected product to preserve unity of invention. Note that PCT Rule 13 does not provide for multiple products or multiple methods.